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ABBREVIATIONS OF TITLES

Abbreviation	*Name of Periodical*
Amer. J. Bot.	American Journal of Botany.
Am. J. Physiol.	American Journal of Physiology.
Annalen	Justus Liebig's Annalen der Chemie.
Ann. Bot.	Annals of Botany.
Ann. Inst. Pasteur	Annales de l'Institut Pasteur.
Arch. Pharm.	Archiv der Pharmazie.
Atti Inst. Bot.	Atti dell'Instituto Botanicó della Universita de Pavia.
Atti R. Accad. Lincei	Atti della Reale Accademia Nazionale dei Lincei.
Ber.	Berichte der deutschen chemischen Gesellschaft.
Biochem. Bull.	Biochemical Bulletin.
Biochem. Z.	Biochemische Zeitschrift.
Bot. Gaz.	Botanical Gazette.
B.P.	British Patent.
Bull. Agric. Intell.	Bulletin of Agricultural Intelligence.
Bull. Wagner Free Inst.	Bulletin of the Wagner Free Institute.
Compt. rend.	Comptes rendus hebdomadaires des Scéances de l'Academie des Sciences.
Compt. rend. Trav. Lab. Carlsberg ...	Comptes rendus des Travaux du Laboratoire Carlsberg.
Dept. of Agr. Travancore, Bull. ...	Bulletin of the Department of Agriculture, Travancore.
Elect. Rev.	Electrical Review.
Electrotech. Z.	Elekrotechnische Zeitschrift.
Elec. Rev. West. Elec.	Electrical Review and Western Electrician.
Elec. World	Electrical World.
Elektrochem. Z.	Elektrochemische Zeitschrift.
Gen. Elec. Rev.	General Electric Review.
Internat. Mitt. Bodenkunde ...	Internationale Mittheilungen für Bodenkunde.
Int. Rev. Agric.	International Review of the Science and Practice of Agriculture.
J. A. C. S.	Journal of the American Chemical Society.
J. Agric. Res.	Journal of Agricultural Research.
J. Agric. Sci.	Journal of Agricultural Science.
Jahr. wissen. Bot.	Jahrbuch für wissentschaftliche Botanik.
J. Amer. Chem. Soc.	Journal of the American Chemical Society.
J. Biol. Chem.	Journal of Biological Chemistry.

Abbreviation	*Name of Periodical*
J. C. S.	Journal of the Chemical Society.
J. Elec. Power and Gas	Journal of Electricity, Power and Gas.
J. I. E. E.	Journal of the Institution of Electrical Engineers.
J. Ind. Eng. Chem.	Journal of Industrial and Engineering Chemistry.
J. Indian Inst. Sci.	Journal of the Indian Institute of Science.
J. Inst. Brewing	Journal of the Institute of Brewing.
J. Pharm.	Pharmaceutical Journal.
J. pr. Chem.	Journal für praktische Chemie.
J. Physiol.	Journal of Physiology.
Mem. Agric. Dept. India, Chem. Ser...	Memoirs of the Department of Agriculture, India, Chemistry Series.
Mikrochem.	Mikrochemie.
Monatsh.	Monatshefte für Chemie und verwandte Theile anderer Wissenschaften.
New Phyt.	New Phytologist.
Pharm. Z.	Pharmazeutische Zentralhalle.
Physiol. Rev....	Physiological Review.
Proc. Bristol Nat. Soc.	Proceedings of the Bristol Naturalists' Society.
Proc. Indian Sci. Congress ...	Proceedings of the Indian Science Congress.
Proc. Roy. Soc.	Proceedings of the Royal Society.
Rec. trav. chim.	Recueil des Travaux chimiques des Pays-Bas.
Rev. gen. Sci. pur. et appl. ...	Revue génerale des Sciences pures et appliquées.
Sci. and Ind.	Science and Industry.
Sci. Proc. Roy. Dublin Soc. ...	Scientific Proceedings of the Royal Dublin Society.
Sci. Progress	Science Progress.
Soil Sci.	Soil Science.
U. S. Pat.	United States Patent.
Woch. Brau.	Wochenschrift für Brauerei.
Z. anal. Chem.	Zeitschrift für analytische Chemie.
Z. angew. Chem.	Zeitschrift für angewandte Chemie.
Zentralbl. Bakt.	Zentralblatt für Bakteriologie.
Z. ges. Brauw.	Zeitschrift für das gesamte Brauwesen.
Z. physiol. Chem.	Hoppe-Seyler's Zeitschrift für physiologische Chemie.

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STUDIES ON THE CHEMICAL COMPOSITION AND PHYSICAL PROPERTIES OF PLANT TISSUE FLUIDS. PART I.

Effects of age and environment on the tissue fluids of French beans (*Phaseolus vulgaris*).

By C. Narasimha Acharya and B. N. Sastri.

In recent years, several attempts have been made to correlate the physiological conditions of plants with the physico-chemical constants of their tissue fluids. Particular attention has been paid to the effects of environment on osmotic pressure (Dixon and Atkins, *Sci. Proc. Roy. Dublin Soc.*, 1912, **13**, 219; 1915, **14**, 374; **15**, 51) and hydrogen-ion concentration (Hurd, *J. Agr. Res.*, 1924, **27**, 725; Rea and Small, *Protoplasma*, 1926–27, **1**, 324; **2**, 428). The study of tissue fluids has also been extended to investigation of plant disease (Contributions to the study of the spike-disease of sandal, *J. Indian Inst. Sci.*, 1928, **11A**, 23, *et. seq.*). No systematic attempt has, however, been so far made to study (*a*) the relation between the chemical composition and the physical constants of tissue fluids derived from different parts of the plant, and (*b*) changes in the composition and properties of tissue fluids with age, nutrition, environment and physiological condition respectively.

Studies of changes in the chemical composition and physical properties of blood under different conditions of health and disease have led to important findings in animal physiology (Myers, *Physiol. Rev.*, 1924, **4**, 274; MacLeod, Physiology and Biochemistry in Modern Medicine, 1926). It may, in like manner, be expected that, detailed study of changes in the kinetic tissue fluids of plants which are responsible for the distribution of nutrients, translocation of metabolism products and the various biochemical changes leading to synthesis and storage of reserve products, or degradation and disposal of waste materials, will lead to useful findings in vegetable physiology.

In the present investigation, an attempt has been made to study the changes in the composition and properties of the tissue fluids from the different parts of *Phaseolus vulgaris*. A few preliminary trials have also been carried out to determine the relation of (*a*) conditions of light and shade, and (*b*) height and general development, on the composition of tissue fluids expressed from the plants.

EXPERIMENTAL.

The seeds were obtained from a local nursery, and sown on 28th April, 1928, in unglazed clay-pots containing a suitable make-up of soil and farmyard manure and provided with bottom drainage. The pots were watered freely from day to day, as is usual with garden crops.

Extraction of tissue fluids.—The plants were removed at intervals of 6 to 7 days in sufficient numbers to provide the required material for each set of determinations. After removal of adhering soil by washing in running water, the plants were divided into leaves, stems and roots. About 150 g. of each tissue were weighed into stoppered bottles, and after addition of a little toluene the bottles were cooled in a freezing mixture of ice and salt and left overnight, when the fluids from the different tissues were expressed by means of a hydraulic press at one ton to the square inch, since preliminary experiments showed that the fluids obtained at pressures of 1, 1·5 and 2 tons were nearly the same in quantity as well as total-solid content; higher pressure than 1 ton was not applied. The fluids thus obtained were centrifuged to remove suspended impurities and preserved in the ice-chest. The following determinations were carried out:—(1) Specific gravity, (2) refractive index, (3) osmotic pressure by calculating measurement of depression in the freezing-point using a micro-Beckmann apparatus, (4) hydrogen-ion concentration by the electrometric method, (5) total solids, (6) loss on ignition at 600°, (7) calcium, potassium and phosphorus in the ash by the A.O.A.C. (*1925*) methods, (8) total nitrogen by Gunning's modification of the Kjeldahl method, (9) total as well as reducing sugars by the method of Davis and Daish (*J. Agric. Sci.*, 1913, **5**, 437) and (10) diastatic activity as described by Sastri and Sreenivasaya (*J. Indian Inst. Sci.*, 1928, **11A**, 23).

Specimens of plants were collected at the following stages:—(1) Emergence of the first leaves (4th May), (2) formation of petioles (8th May), (3) appearance of the nodules on most of the roots (13th May), (4) formation of flower buds (21st May), (5) opening of the first flower (26th May), (6) formation of the pod (1st June), (7) first appearance of the seeds (6th June), (8) maximum development of the first pod (10th June), (9) ripening of seed and (10) harvest of beans (18th June).

Meteorological observations (Table I) during the period of investigation show that although the average minimum temperature remained about the same throughout, the corresponding figures for maximum temperature and hours of sunshine tended to decrease, and the relative humidity to increase towards the later stages. The periods

of flowering and seeding were marked by somewhat dull weather which should have led to the slackening of those two processes. It may be inferred that with a hot and dry weather as prevailed in the beginning, the changes corresponding to the formation and the maturation of the bean would have proceeded more rapidly than observed in the present investigation, although no doubt the chemical mechanism of the processes would have been the same.

TABLE I.

Meteorological observations from 23rd April to 21st June.

Period Week ending on	Max. temp.	Min. temp.	Hours bright sunshine	Rainfall, inches	Relative humidity, percentage
28th April	97·4	71·9	9·3	...	41
4th May	98·4	72·5	9·5	0·002	47
10th ,,	94·9	70·6	7·8	0·015	56
16th ,,	95·2	69·6	10·1	0·230	49
22nd ,,	96·6	71·9	9·1	0·048	52
28th ,,	94·3	69·3	10·2	0·062	50
3rd June	94·1	69·8	10·0	0·202	51
9th ,,	84·0	67·6	3·6	0·015	65
15th ,,	85·2	67·9	4·2	0·087	68
21st ,,	87·3	66·6	6·3	0·012	58

General observations.—With the growth of the plant, the shoot lengthened from 6 cm. on the 7th day after sowing to 30 cm. at the stage of maximum development of the pod; there was no increase during the ripening of the seed (10th to 18th June). A similar observation was made with regard to the root-length measured from time to time. The average total weight of the plant increased from 1·65 g. when the first leaves emerged to 19·4 g. when the pod was fully formed (10th June) but fell to 17·6 g. when the seed was fully ripe (18th June). Changes in the weight of leaf and stem respectively were similar to those of total weight, but the weight of the root increased right up to the end. The yield of tissue fluid from the leaf was more or less constant (85—89 per cent.) up to the stage of seed development but showed a slight decline (76 per cent.) as the seed ripened: the yields from stem and root—decreased to a greater extent with the age of the plant, and fell from 84 to 57 per cent. in the case of the stem and from 83 to 66 per cent. in that of the root.

Specific Gravity, Refractive index, Osmotic pressure, Total solids and Mineral ash.

The figures (I—IV) show that tissue fluids from different parts exhibit progressive but similar variations at various stages during the growth of the plant. The curves are generally double topped ones: and show either two maxima or two minima corresponding to the stages of development of flower buds and formation of seeds respectively.

As the plant grows the tissue fluids, thin and dilute in the early stages, become richer in both mineral and organic matter, showing a progressive increase in specific gravity, osmotic pressure, total solids and mineral ash content, till the period of flowering. When flower buds are formed however, there is a drop in the concentration of the sap, the plant foods being transported to the centre of reproduction. The curves show a minimum immediately after the flowers open, and maintain a low level till the seeds are ripe. Such a stage may be expected to represent a period of low vitality in the plant. It is probably on this account that a plant is particularly susceptible to disease at the flowering stage.

The leaf-sap has generally a higher specific gravity and contains more of total solids than the stem. The fluid from the root gives invariably lower values than those from the leaf and stem for all attributes mentioned above, thereby suggesting that it is more dilute than the others.

The following were the ranges of the refractive indices of the tissue fluids from the different parts of the plant, measured at 26° :— Leaf, 1·3375—1·3402; stem, 1·3369—1·3408; and root, 1·3356—1·3368. There was generally a close correlation between the refractive indices and the corresponding total solid contents.

Reaction (Fig. V).—The hydrogen-ion concentration of the tissue fluids changes progressively during growth. Thus the P_H values for the fluid of the leaf ranges from 5·66 to 6·21, that of the stem from 5·6 to 5·9 and that of the root from 5·85 to 6·29. In all cases maximum acidity is reached at the time of flowering after which there is a steady return to the initial reactions.

Nitrogen (Fig. VI).—In the early stages, when vegetative growth is rapid, the sap is comparatively rich in nitrogen. With the approach of the flowering stage, the nitrogen content decreases rapidly and reaches a minimum when the first flower buds appear. The low level of nitrogen continues during the seeding stage, but tends to rise again as the plant ages. This observation is in accordance with those of

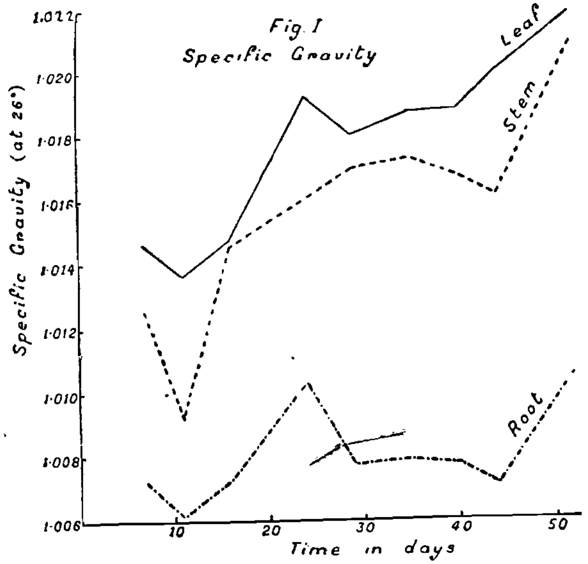

Fig. I
Specific Gravity

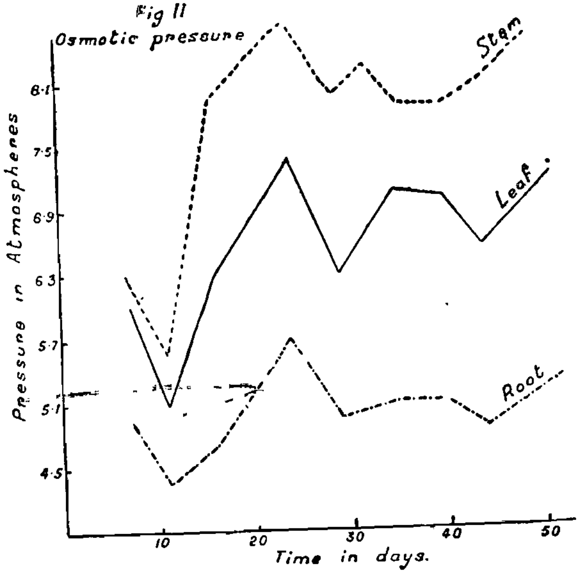

Fig II
Osmotic pressure

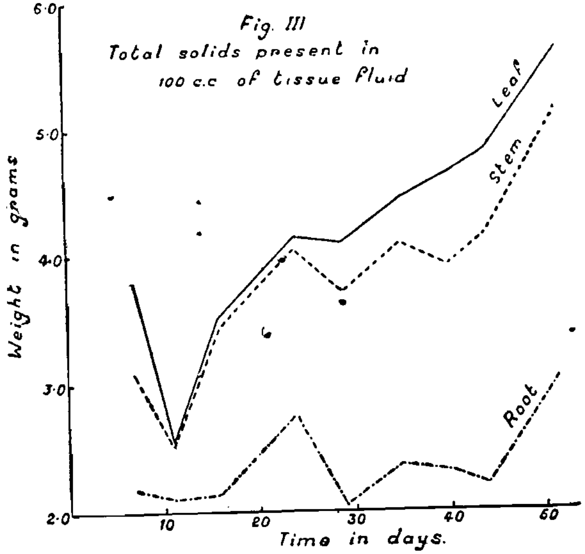

Fig. III
Total solids present in
100 c.c of tissue fluid

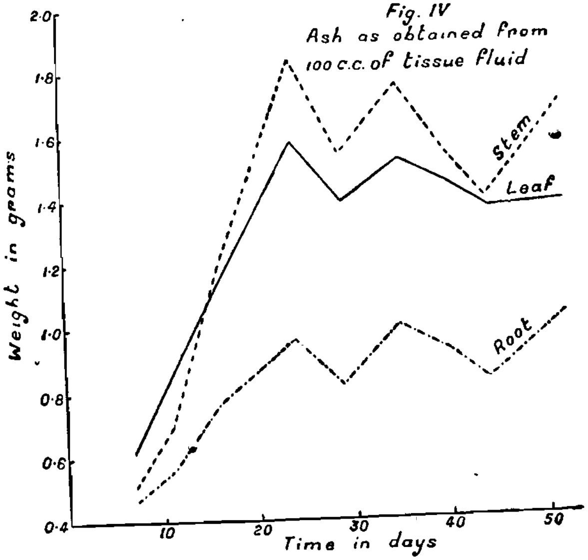

Fig. IV
Ash as obtained from
100 c.c. of tissue fluid

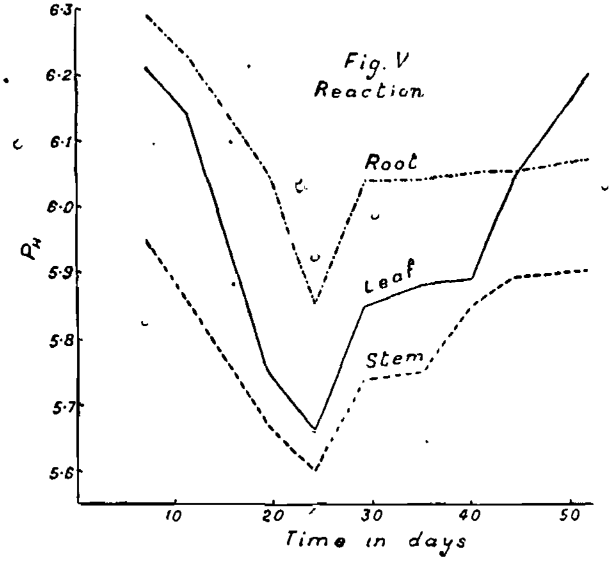

Fig. V
Reaction

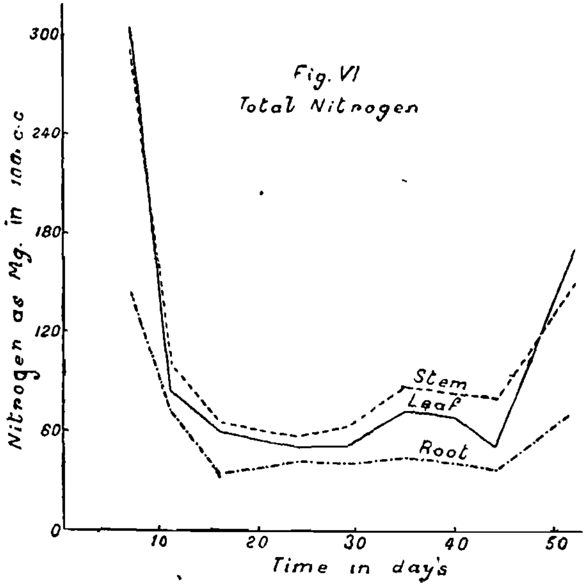

Fig. VI
Total Nitrogen

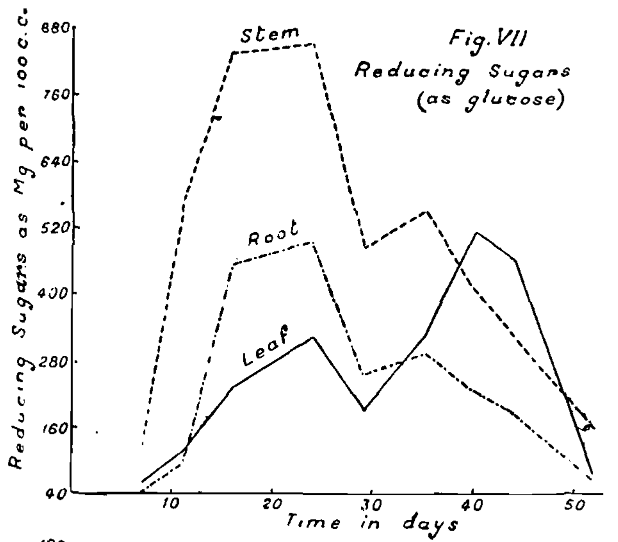

Fig. VII
Reducing Sugars
(as glucose)

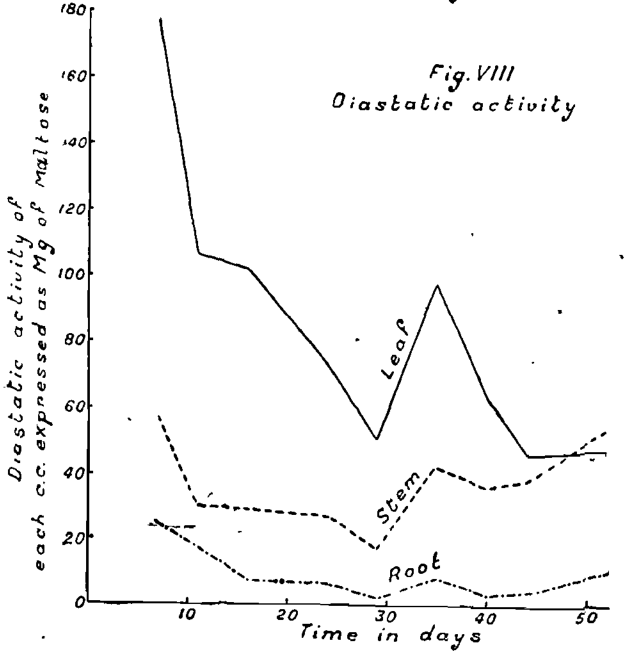

Fig. VIII
Diastatic activity

Hicks (*New Phyt.*, 1928, **27**, 108), Gericke (*Soil Sci.*, 1922, **13**, 135) and others.

Sugars.—Reducing sugars account for nearly all the total sugar present in the different tissue fluids, so these only are presented in Fig. VII. As contrasted with nitrogen, the sugars increase steadily in the fluids from the different parts of the plant until the flowering stage is reached. Decrease then sets in, and excepting a slight tendency to increase at the time of seed development, the sugars diminish until they become almost negligible at the time of harvesting.

Diastatic activity (Fig. VIII).—Diastatic activity is prominent in the fluid from the leaves particularly in the early stages, but declines rapidly: a brief pause is noticed at the time of flowering, but soon the decline is resumed until the activity at harvesting becomes less than one-third of the original. The activity of the fluid from the stem is generally lower than that from the leaf, and is not so much affected by the age and condition of the plant. The diastatic activity of the fluid from the root is generally very much less than that of the other tissues, and is almost negligible at the time of flowering and seeding.

Tall and stunted plants.—Although all the plants belonged to the same variety and were raised under identical conditions, it was noticed that some plants were taller and better developed than others. In view of the possibility of chemical and physico-chemical attributes of such plants differing from each other, the determinations described above were carried out with them.

It was observed that rapid vegetative growth was accompanied by lower specific gravity, refractive index and total solids in the fluids from the stem and the root, while in the case of that from the leaf the reverse was true. The data for osmotic pressure, nitrogen, ash content and diastatic activity of the fluid from all parts of the tall plants are also lower than the corresponding figures for the stunted ones.

Effect of darkness.—To study the effect of darkness and consequent arrest of photosynthesis, representative plants of different ages were stored in the dark for 24 hours and their tissue fluids then examined in the manner described already.

It was observed that the fluid from the leaf was the first to suffer. Specific gravity, refractive index and total solids, originally high, became very much less as the result of storage in darkness. The reaction was also affected. Thus, whereas the reactions of the sap from leaf, stem and root were originally P_H 5·88, P_H 5·75 and P_H 6·04 respectively, they became P_H 6·71, P_H 6·44 and P_H 6·2 in darkness. This observation is in accordance with the findings of

Garner, Bacon and Allard (*J. Agric. Res.*, 1924, **27**, 119), who noted that decrease in the length of daily illumination is marked by corresponding reduction in the active acidity of the sap.

It was also noted that decrease in the carbohydrate content of plants kept in darkness was accompanied by accumulation of nitrogen in the leaves.

Composition of ash (Fig. IV).—There is considerable variation in the mineral ash contents of the saps expressed at various stages in the life of the plant, ranging from about 0·46 g. per 100 c.c. of the fluid from the root to about 1·71 g. in that from the stem. The composition of the mineral matter is fairly uniform. Potassium oxide forms the major constituent in all cases and accounts for 30 to 50 per cent. of the total mineral matter. The highest percentage is found in the stem, which is also the richest in carbohydrates.

The figures for calcium (as CaO) show more variation. There is but little calcium in the root sap, at no time more than 6 per cent. of the total ash. The percentages in tissue fluid from the stem range from 6 to 12 per cent. while those for the fluid from the leaf increase from about 7 per cent. in the earlier stages to about 20 per cent. at the time of harvest.

The amounts of phosphorus present in the fluids from the different parts are invariably low, being at no time more than 3 per cent. of the total ash. It was also noted that the phosphorus and potash contents of fluids from plants kept in darkness were higher than those from illuminated plants.

DISCUSSION.

The foregoing observations show clearly that the tissue fluids of *Phaseolus vulgaris* undergo changes in chemical composition and physical properties which correspond to different stages in the life of the plant.

As the plant develops from the seedling stage, its roots collect minerals from the soil and the leaves build up sugars and other photosynthetic products, which are subsequently translocated to the other parts of the plant. The total solids, the sugars, and the minerals in the tissue fluids from the different parts of the plant increase in consequence and continue to do so until the flowering period is reached. After that stage, however, though the assimilation of nutrients through the roots and photosynthetic activity still continue, translocation of

carbohydrates and minerals into the flowers proceeds so rapidly that the quantities of those substances in the tissue fluids from different parts of the plant are considerably lowered. The decrease in the different constituents continues throughout the flowering and the seeding stages, after which there is a tendency for the original composition to be restored.

By showing a continuous decrease throughout the life of the plant, nitrogen of the tissue fluids appears to be an exception to the observations described above : but considering that the total nitrogen in the whole plant increased almost to the day of harvest, it may be inferred that the apparent diminution in the nitrogen percentage of the tissue fluids is due to the proportionately greater photosynthetic activity. Nitrogen is being assimilated from the soil as well as fixed from the atmosphere throughout the life of the plant. The quantities of nitrogen thus passing into the tissue fluids are, however, proportionately less than the carbohydrates and other products of photosynthetic activity in the fluids at the same time. The decreased proportion of nitrogen is therefore due not to diminished intake, but to disproportionately greater photosynthetic activity, throughout the life of the plant.

The change in reaction at the time of flowering may be attributed to rapid translocation of the different sugars and mineral salts from the various parts of the plant to the flower buds. A poorly buffered condition results and a somewhat acid reaction is observed. After the flowers are formed, there is less translocation from the different parts with the result that increasing amounts of the different buffering materials collect in the tissue fluids and, in consequence, the original reaction is rapidly attained.

The diminution observed in diastatic activity, particularly in the leaf tissue fluid, suggests that as the plant ages there is less and less hydrolysis of starch taking place. Since, however, the translocation of carbohydrate materials to the different parts, particularly to the flowers and the seeds takes place throughout the life of the plant, it may be inferred that such changes are probably not preceded by hydrolysis of starchy materials. The rapidity of the changes, particularly at the flowering stage, suggests that the photosynthesised materials are carried by the tissue fluids from the leaf and, to a smaller extent, from the other parts directly to the flower buds.

The study of the composition of the tissue fluids from the tall and the stunted plants shows that there is greater photosynthetic activity in the former than in the latter. The occurrence of considerably greater percentages of minerals and nitrogen in the tissue fluids from the stunted plants is obviously due to diminishing amounts of water and photosynthesised materials passing into them. The observation also

suggests that the stunted condition is due to some of the plants having received more nitrogen and minerals in the earlier stages than was really needed and in consequence, 'lodging', a phenomenon commonly observed in the field crops whenever soluble plant nutrients are supplied in excess.

The composition of the fluids from plants kept in darkness has been affected considerably. The respiration of the plant proceeds throughout the period of storage in darkness resulting in the utilisation of the mobile carbohydrates in the tissue fluids. There is no photosynthetic activity to make up for the loss, and hence follows the diminution in the various constituents of all tissue fluids. The hydrogen-ion concentration is also affected by steady disappearance of the different materials, and probably also by development of certain organic acids. The mineral and nitrogen contents also decrease as the result of storage in darkness, showing that in absence of photosynthesis, supply of the nutrients essential to the active functioning of the roots in the absorption of minerals, and of the root-nodule bacteria in the fixation of atmospheric nitrogen, has been retarded.

SUMMARY.

The more important physical properties and chemical constituents of the tissue fluids from different parts of *Phaseolus vulgaris* at various life-stages were determined and their relation to age and environment studied.

The observations show that the tissue fluids become enriched with minerals and the different products of photosynthesis up to the stage of flowering, when rapid translocation of the different constituents to the flower buds take place. After that period, although the assimilation of minerals from the soil, the fixation of nitrogen from the air and the photosynthetic processes continue at the same rate, yet the translocation of the various substances into the flowers and seeds proceeds so rapidly that the tissue fluids in the other parts are rendered comparatively weaker in the various constituents almost up to the time of harvest.

The tissue fluids from tall and stunted plants of the same age were examined. The 'lodging' observed in the case of the latter was found to be associated with accumulation of nitrogen in the leaf and low water content of the tissue fluids from the different parts of the plant.

Examination of plants stored in darkness showed that owing to prolonged respiration of carbohydrate materials without compensation

by photosynthesis, the tissue fluids from the different parts are rendered poor in carbohydrates and solid matter in general. Owing to inadequacy of carbohydrate nutrition, nitrogen fixation in the nodules and assimilation of minerals by the roots are distinctly affected.

In conclusion, we beg to acknowledge our grateful thanks to Dr. V. Subrahmanyan for his kind interest in the progress of the work and suggestive criticism.

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THE PHOTOCHEMICAL OXIDATION OF ORGANIC HYDROCARBONS.

Part II. Toluene, and the Oxidation of Benzaldehyde.

By C. J. Kothari and H. E. Watson.

In the first part of this series (Sudborough, Watson and Narayanan, *J. Indian Inst. Sci.*, 1925, 8A, 1) an account was given of preliminary experiments on the oxidation of toluene by oxygen under the action of ultraviolet light in presence of anthraquinone as a catalyst. Further experiments have now been conducted with toluene, and as benzaldehyde was found to be one of the products of oxidation, an investigation has been made of the oxidation of benzaldehyde alone and in solution. It has been shown that the reactions are complex, and since the experimental difficulties of uniformly exposing solutions to the action of a gas are considerable, and the velocity is influenced by minute traces of impurities, it has been difficult to obtain strictly quantitative values in all the experiments. Several facts have however been brought to light, and further work on the subject being postponed, it appears desirable to record the results so far obtained. Before the mechanism of the reactions can be elucidated much more detailed work will be required.

TOLUENE.

References to previous work upon toluene have already been given in part I. To these may be added a short paper by John (*Ber.*, 1925, 58, 1563), in which it was shown that the rate of formation of benzoic acid in sunlight with anthraquinone as catalyst depended upon the elevation above sea-level, evidently owing to the increased amount of ultraviolet light at higher altitudes.

EXPERIMENTAL.

The general arrangements were the same as those described in part I, the reaction mixture being placed in a closed distilling flask connected with an oxygen reservoir and shaken in close proximity to a mercury arc lamp. The products were analysed for benzoic acid as before and in addition, a more detailed examination according to the

method of Eckert (*Ber.*, 1925, **58**, 1563) was made to detect other substances which might have been formed in the reaction. Benzaldehyde and a substance which appeared to be isohydrobenzoin were isolated but hydrobenzoin, benzil and dibenzyl found by Eckert could not be identified. Kailan (*Monatsh.*, 1919, **40**, 445) mentions formic and oxalic acids as reaction products, the former occurring in the aqueous layer in a quantity approaching that of the benzoic acid, but his deductions were based mainly on conductivity measurements and in neither case does he appear to have isolated the acid or a derivative ; he states, however, that the solution reduced ammoniacal silver nitrate solutions. In our experiments no solution was obtained exhibiting this property but on the other hand the aqueous liquors gave the usual tests for hydrogen peroxide. In addition, the amount of permanganate decolorised by the solution corresponded with a quantity of formic acid much greater than the total acidity of the solution, which was mainly due to benzoic acid. The presence of hydrogen peroxide therefore seems highly probable and its formation may account for the greater rates of oxidation observed in presence of a large proportion of water ; Kailan, reasoning from experiments in the absence of toluene, considered the formation of hydrogen peroxide unlikely. It is possible that both series of observations are correct since Kailan's experiments were made without a catalyst and the mechanism of the reaction may be different in the two cases.

Preliminary experiments showed that a mixture of toluene, anthraquinone and water underwent no appreciable oxidation in the dark in 3 months at room temperature or in 133 hours at 60°. Illumination with a 1 kilowatt metal filament lamp was also without effect. On the other hand, reaction was found to proceed at very nearly equal rates in quartz and glass vessels under the influence of the radiation from a quartz mercury arc lamp, a fact previously noted by John (*loc. cit.*). The later experiments were consequently conducted in glass vessels.

The Course of the Reaction.

Three flasks each containing 50 g. toluene, 5 g. water and 2·5 g. anthraquinone were exposed simultaneously to light from the arc at a distance of about 15 cm. with continuous shaking, a slight positive pressure of oxygen being maintained. The experiment was stopped during the night and at the end of each day's run ; after cooling and allowing the mixture to separate into two layers, 5 c.c. of the toluene solution were withdrawn from one of the flasks and titrated. When the contents of one flask were exhausted samples were taken from the next. Table I shows the total quantity of benzoic acid in the toluene layer at the end of each day's exposure and the number of milligrams of acid produced per hour for the corresponding period.

TABLE I.

Rate of formation of benzoic acid from toluene.

Flask 1.			Flask 2.			Flask 3.		
Time hours	Total acid g.	mg./hr.	Time hrs.	Total acid g.	mg./hr.	Time hrs.	Total acid g.	mg./hr.
9·7	0·014	1·4	62	0·71	(11)	111	2·00	(18)
16·0	0·020	1·1	69	0·84	27	118	1·81	21
22·3	0·026	1·6	76	0·95	27	125	1·78	23
28·3	0·034	2·5	83	1·00	21	132	1·73	23
35·0	0·052	4·3	90	0·92	23	139	1·67	24
41·7	0·066	3·7	97	0·93	21	146	1·55	20
49·2	0·080	4·4	104	0·86	14	153	1·40	19
...	...	...	111	0·77	14	...	...	...
Total acid formed	0·163	...	...	1·75	...	...	3·22	...

These figures show that initially the reaction is very slow but the rate increases steadily and tends to approach a constant value. Flask 2 was a little closer to the light than flask 3 and this may partly account for the somewhat higher rate in the former case when the contents were first examined. As the volume of toluene was diminished by withdrawing samples, the rate of acid-formation fell, an effect best seen with flask 2. The quantity of acid in flask 2 at the end of 62 hours is evidently much greater than the total amount which would have been formed in flask 1 if the reaction had been allowed to continue another 13 hours and the quantity in flask 3 after 111 hours exposure is greater than the total amount formed in flask 2 in this period. The explanation is that the reaction does not take place solely at the glass surface as appears to be the case with benzaldehyde ; but that oxidation occurs throughout the liquid and depends on the total volume. As water is always present, it is probable that the extent of the water-toluene interface is the controlling factor. It may be mentioned that the 'total acid' given in the table is the sum of the acid in the flask and the quantity removed as samples. In some cases the sample contains more acid than is formed during the subsequent period of exposure so that the amount in the flask diminishes.

After 153 hours' exposure, 40 c.c. of fresh toluene and the corresponding quantity of anthraquinone were added to 13 c.c. of toluene and the water from flask 3, and the exposure was continued. The

rate of acid formation was only 6 mg./hr. for the first 7 hours increasing to 10 and 12 mg. for the next two similar periods. This rate is much greater than the initial figures for flask 1 and renders it improbable that the slow oxidation when the reaction is first started is due to an anticatalyst contained in the toluene which is slowly destroyed. It appears more likely that intermediate products are formed and thus, on dilution, the rate is lowered until they again attain their maximum concentration. The possibility of benzaldehyde being such an intermediate product was tested by adding 0·3 c.c. to a fresh reaction mixture, but no initial acceleration was observed; in another experiment, the residue left in a flask which had been exposed to light for a prolonged period and from which the toluene had been distilled was added to fresh toluene, also without any result. It follows therefore that the accelerator is either readily volatile or is destroyed by heat. A peroxide would satisfy these conditions.

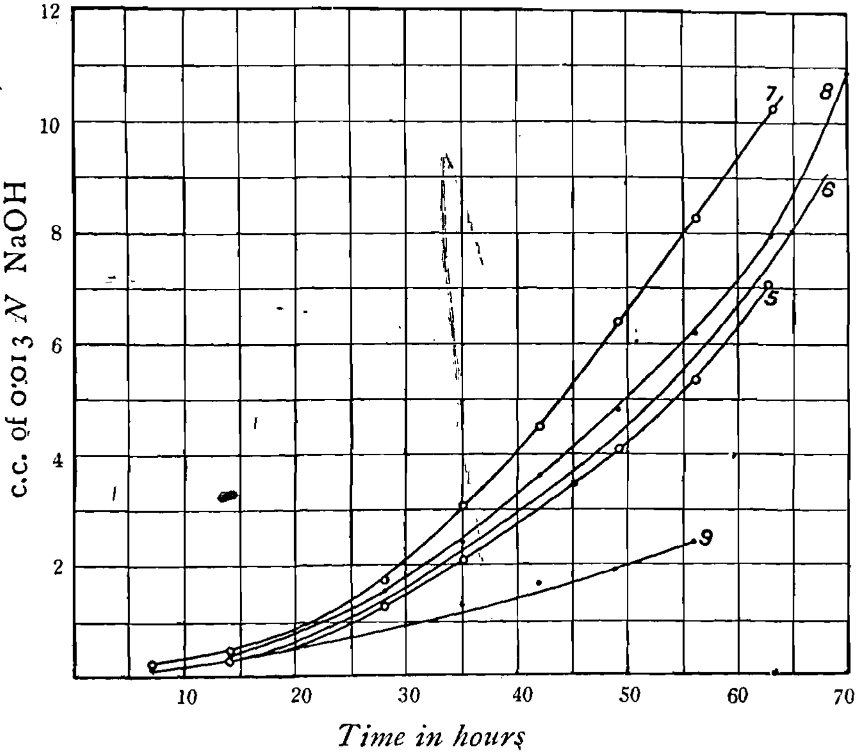
The fall in reaction-rate may be partly due to the accumulation of benzoic acid. To examine this possibility, an experiment similar to the preceding was conducted and the concentration of acid determined at regular intervals. After 80 hours' exposure, when the concentration was 3 g. in 100 c.c., the benzoic acid was removed by extraction with sodium carbonate and the mixture again exposed. The curve connecting the rate of increase of concentration with time showed no discontinuity, indicating that at the concentration examined the effect was negligible.

The previous experiments were made with 5 per cent. of catalyst, the quantity recommended by Eckert. As this proportion was not completely soluble it appeared likely that a smaller amount would be equally efficacious, and so a series of experiments was conducted in which varying proportions of anthraquinone were added to a mixture of 50 g. of toluene and 5 c.c. of water. The results expressed as c.c. of 0·013 *N* sodium hydrate required to neutralise 5 c.c. of the solution are shown in fig. I, all samples being withdrawn at equal intervals. The percentage of catalyst used in experiments 5 to 9 was 4·3, 2·5, 1·0, 0·5 and 0·05, respectively. The most rapid formation of acid is with 1 per cent. of catalyst. This stage in the experiments was reached in the early part of the year, the period of minimum cloudiness and the opportunity was taken to make some measurements in sunlight.

Oxidation by Exposure to Sunlight.

The method adopted was the same as with the mercury arc, the whole apparatus being placed out of doors in an exposed position. The usual temperature attained was about 50°, somewhat lower than in the case of the arc.

FIG. I.



c.c. of 0·013 N NaOH
12
10
8
6
4
2
10
20
30
40
50
60
70
Time in hours
7
8
6
5
9

The effect of adding quinones and uranium salts, which are known to act as photo-catalysts in some reactions, was first tried. It has already been shown by Benrath and Meyer (*Ber.*, 1912, **45**, 2707) that phenanthraquinone accelerates the oxidation of toluene, but becomes oxidised to diphenic acid and hence cannot be used for long periods. No experiments appear to have been made with other quinones except anthraquinone and consequently we have tried benzoquinone and camphorquinone. The former was found to decompose rapidly yielding a dark solution in about 30 minutes, while the latter substance also changed, the originally yellow solution becoming colourless in 3 to 4 hours.

Table II shows the c. c. of 0·1 *N* sodium hydrate solution required to neutralise a mixture of 25 g. toluene and 12·5 g. water after exposure. The weight of catalyst in expts. 1 and 2 was 0·5 g., and in expt. 3, 0·3 g.

TABLE II.

Expt. No.	Exposure hours	c. c. 0·1 *N* NaOH for whole mixture		
		Uranium nitrate	Uranium acetate	Anthraquinone
1	24	21·3	30·1	80·2
2	47	26·9	23·7	226·0
		Benzoquinone	Camphorquinone	
3	70	5·8	19·2	266·4

The experiments were conducted at different times and are thus not comparable with each other owing to variations in the light intensity. The uranium salts have some catalytic action, but the results are irregular and the amount of oxidation does not appear to increase with the time. Benzoquinone and camphorquinone as anticipated give rise to very little oxidation, the acid being in all probability formed for the most part by direct reduction of the catalyst. Hydrogen poroxide was not detected in any of the solutions except when anthraquinone was used, pointing to difference in the mechanism of oxidation.

The proportion of toluene oxidised in these experiments when anthraquinone was used as catalyst (8·3 and 9·8 per cent. in experiments 2 and 3, respectively) was larger in proportion to the time than in any of those with a mercury arc as the source of light. For example, in the preliminary experiments with quartz flasks, 12·2 per cent. was converted in 200 hours and in experiments recorded later the amounts

in 70 hours were 2·6 and 4·3 per cent. In these only 10 per cent. of water was used. In the previous series of experiments with 25 per cent. of water the highest value in 200 hours was 11·6 per cent.

Although experiments had shown that small quantities of benzaldehyde did not influence the rate of reaction, this fact would not disprove its formation as an intermediate product in the scheme,

Toluene $\rightarrow$ benzaldehyde $\rightarrow$ benzoic acid.

If this were the case, pyrogallol, which is an inhibitor of the second reaction, should reduce the rate of formation of benzoic acid from toluene. To test this hypothesis three solutions were made each containing 25 g. toluene, 12·5 g. water and 0·3 g. anthraquinone; pyrogallol was added to two, and all three were exposed simultaneously to sunlight for 34 hours with continuous shaking. The acid formed was then titrated with 0·1 *N* sodium hydroxide solution with the following results :—

Pyrogallol, g.	...	0·0	0·005	0·01
c. c. 0·1 *N* NaOH	...	136	148	155

It appears from these figures that pyrogallol, instead of retarding the reaction, slightly accelerates it and consequently the suggested mechanism with benzaldehyde as an intermediate product is probably incorrect unless the rate of oxidation of benzaldehyde is so great that even in presence of pyrogallol it is large compared with the rate of oxidation of toluene.

It is unfortunately not easy to study the oxidation of very dilute solutions of benzaldehyde and so an examination of the reaction under other conditions has been made. This has shown that toluene itself exerts a retarding influence, thereby introducing a further complication, and so far, it has not been possible to arrive at a definite conclusion.

THE OXIDATION OF BENZALDEHYDE.

The oxidation of benzaldehyde by oxygen has been studied by Schönbein (*J. pr. Chem.*, 1858, **74**, 328; 1861, **84**, 406; 1868, **105**, 226), Bach (*Compt. rend.*, 1897, **124**, 951) who showed the presence of peroxides in the oxidised mixture, Engler and Wild (*Ber.*, 1897, **30**, 1677), and Nef (*Annalen*, 1897, **298**, 280) who were of opinion that benzoyl peroxide in addition to hydrogen peroxide was present, Staudinger (*Ber.*, 1913, **46**, 3530) and more recently by Jorrisen and his collaborators (*Rec. trav. chim.*, 1926, **45**, 245; 1927, **46**, 42). These authors actually isolated from the reaction products the benzoyl

hydroperoxide, $C_6H_5\cdot CO\cdot O\cdot OH$, prepared by Baeyer and Villiger (*Ber.*, 1900, **33**, 1582), but later found that the course of the reaction depended upon the solvent. Kailan (*Monatsh.*, 1912, **33**, 1305) measured the reaction velocity in solutions of benzene and alcohol under the influence of light from a quartz mercury arc, and found the temperature coefficient to be small.

Moureu and Dufraisse (*Compt. rend.*, 1922, **174**, 258; 1924, **178**, 824, 1861; 1924, **179**, 237; 1926, **183**, 408) have made a careful study of the action of inhibitors in very weak light and have explained it by supposing the formation of peroxides which mutually destroy each other, regenerating the original compounds.

While the present work was in progress an important contribution was made to the subject by Reiff, (*J. Amer. Chem. Soc.*, 1926, **48**, 2893) who showed the part played by the surfaces with which the aldehyde was in contact, particularly when they were wet. Still more recently Backstrom (*J. Amer. Chem. Soc.*, 1927, **49**, 1460) has made measurements of the quantum efficiency and deduced that the photochemical reaction is a chain reaction; moreover, because inhibitors have a similar action on both thermal and photochemical reactions, he concludes that the former is a chain reaction as well. Unfortunately his inhibitors did not include those which have been most commonly studied. None of these papers can be said to afford conclusive evidence regarding the reaction and our experiments tend to show that a number of factors must be taken into account.

EXPERIMENTAL.

Benzaldehyde was distilled under reduced pressure and stored in the dark in small sealed tubes. The early experiments were made with a sample which was subsequently found to contain chlorine compounds; later this was replaced by a chlorine-free product made from toluene. The benzene and toluene used as solvents were carefully purified.

Preliminary experiments made by bubbling oxygen through a 33 per cent. solution of benzaldehyde in toluene and measuring the amount absorbed showed that the reaction took place in the dark and had an appreciable temperature-coefficient; it was also retarded if the solution is in contact with rubber. The results were irregular and it was evident that careful control of temperature and a fixed rate of bubbling were essential if consistent values were to be obtained. Immersion of the reaction vessel in a thermostat was found unsatisfactory as it was difficult to keep the water perfectly clean. The gas

circulating devices of Pearson and Thomas (*J.C.S.*, 1925, **127**, 2450) and of Chatterji and Finch (*J.C.S.*, 1925, **127**, 2464) were tried but did not satisfy the requirements of these experiments. The arrangement finally employed is shown in fig. II. A 50 c.c. jacketed gas burette *A*, was used to hold a supply of oxygen and measure its volume. The gas was passed through the reaction vessel *B* by means of a circulating pump consisting of two mercury reservoirs *CC* connected by rubber tubing, one of which could be raised and lowered at constant intervals by means of an eccentric attached to gearing driven by a motor. The valves *DD* consisted of globules of mercury at the junction between a capillary and a wider tube. Gas could bubble through the mercury in one direction but was prevented from passing the other way by the rise of the mercury in the capillary tube. These valves were very satisfactory and did not leak. The delivery of the pump could be varied by changing the lift or altering the rate. As used, at 20 revolutions per minute the volume of gas circulated was 2 litres per hour. The water in the gas burette formed a buffer tending to equalise pressure variations and the result was a nearly steady stream of gas through the reaction liquid. The reaction vessel *E* was immersed in a vessel of distilled water with flat sides, the temperature being maintained constant by a coil through which was circulated water from a thermostat. For illumination, a 100 c.p. pointolite lamp run with a constant current of 1·2 amps. at a distance of 19 cm. from the reaction vessel was used, the light passing through a filter containing water to cut off the heat rays.

When starting an experiment the apparatus was first filled with oxygen from a gas holder and the air displaced. Gas was then admitted to the gas burette and the circulating pump started. At 20 or 30 minute intervals it was stopped and the volume read; when the gas burette was empty fresh oxygen was introduced from the gas holder. This sometimes caused an irregularity in the next reading particularly with benzene as solvent owing to the fresh gas not being saturated with the vapour. It was found that if the gas was bubbled through the solvent before admission the irregularity was minimised although not always entirely eliminated.

Preliminary experiments showed that the rate of absorption of oxygen fell in an unexpected manner as the reaction progressed and it was suspected that the benzoic acid produced by the reaction might have an inhibiting effect. Several observations were made for solutions containing benzoic acid and the results are shown in table III which gives the volume of oxygen (c.c. at N.T.P.) absorbed in successive periods of 30 minutes by a toluene solution containing 33 per cent. of aldehyde.

FIG. II.

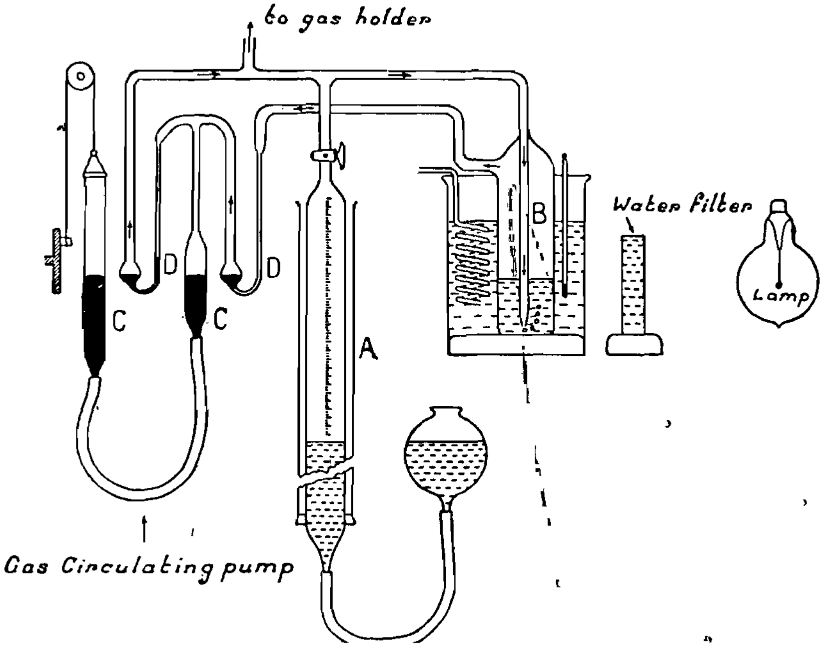

to gas holder
B
Water filter
Lamp
D
D
C
C
A
Gas Circulating pump

TABLE III.

Absorption of oxygen in 30 minutes by 33 per cent. solution of benzaldehyde in toluene.

Expt. No. 16	18	20	27
No acid	0·9 g. acid I	0·9 g. acid II	0·9 g. acid III
15·1	22·7	14·9	17·8
15·1	17·5	13·9	18·0
14·9	14·4	11·0	14·9
12·0	12·3	9·7	11·3
12·1	10·5	8·2	9·8
11·4	10·0	7·5	9·3
10·2	10·0	7·3	8·6
10·0	9·0	6·5	...
100·8	106·4	79·0	...

The quantity of acid formed at the end of expt. 16 was approximately 0·9 g. and this amount was added in each of the other three experiments. If the acid had a retarding effect, the initial rate of absorption in these should have been the same as the final rate in expt. 16, but this was not so. Acid I was a fairly pure commercial acid and its effect was to accelerate the reaction initially although the relative retardation occurring subsequently was greater than in expt. 16. Acid II was recovered from previous oxidation experiments and in its presence the rate was definitely retarded, particularly in the later stages. Acid III was a specially purified sample prepared from toluene.

These results might be explained by the assumption that the benzoic acid used in expts. 18 and 27 and the benzaldehyde itself, contained as impurity an accelerator which was gradually destroyed as the reaction proceeded. Subsequent experiments, however, have failed to confirm this hypothesis. A comparison of expts. 16 and 20 indicates that pure benzoic acid has a retarding influence especially at higher concentrations, but it is evident that the rapid fall in reaction velocity is not due solely to the accumulation of the reaction product.

The sample of benzaldehyde used in the experiments just described as well as the first two samples of benzoic acid were

found to contain traces of chlorine. It was therefore decided to try the effect of adding to a chlorine-free specimen of benzaldehyde small quantities of such chlorine compounds as might occur as impurities. Table IV shows the quantities of oxygen absorbed in successive periods of 30 minutes by a 50 per cent. solution of benzaldehyde in toluene at 30° with different additions, the illumination and rate of bubbling being as before.

TABLE IV.

Absorption of oxygen in 30 minutes by 50 per cent. solution of benzaldehyde in toluene.

Expt. No.	Substance	Oxygen absorbed						Total, 3 hours
12	Benzaldehyde impure ...	57·9	49·3	41·7	35·0	31·5	24·5	239·9
4	Benzaldehyde pure (*a*) ...	47·0	42·1	36·2	27·6	26·5	22·4	201·8
5	*do.* + 0·05 g. *o*-chlorobenzoic acid ...	50·2	41·1	32·7	28·4	25·5	22·1	200·0
7	*do.* + 0·05 g. *m*-chlorobenzoic acid ...	45·6	51·3	43·7	38·7	28·9	25·0	233·2
8	*do.* + *do* *do.* ...	42·3	53·2	44·7	32·4	26·9	22·0	221·5
10	*do.* + 0·02 g. *p*-chlorobenzoic acid ...	44·2	38·6	32·2	28·5	26·0	22·2	191·7
17	Benzaldehyde pure (*b*) ...	43·7	39·0	31·1	26·5	24·2	20·7	185·2
16	*do.* + 0·1 g. benzyl chloride ...	35·1	30·8	28·8	25·2	21·0	19·5	160·4
18	*do.* + 0·05 g. *do.* ..	42·1	35·5	30·3	27·0	...	...	...
21	*do.* + 0·2 g. benzyl chloride ...	36·3	31·3	25·6	21·3	14·1	15·0	143·6
20	*do.* + 0·1 g. *do.* ...	38·6	30·2	23·5	21·0	19·5	17·9	150·7
23	*do.* + 0·2 g. benzotrichloride...	32·2	27·5	25·0	23·4	18·0	14·0	140·1
24	*do.* + 0·02 g. *do.* ...	48·3	34·3	32·4	28·6	27·3	27·3	198·2
30	*do.* + 0·1 g. anthraquinone ...	61·6	49·2	45·1	40·6	37·5	33·2	267·2

Examination of these figures reveals the fact that in spite of all precautions, the rate of oxidation is irregular and the experimental error large; consequently too much reliance must not be placed upon individual results. Certain conclusions can, however, be drawn and of these the most obvious is the diminution in the rate of absorption to approximately half its original value in 3 hours, the decrease in concentration for the absorption of 200. c. c. being only 16 per cent. It will be shown later that the rate of oxidation diminishes much more rapidly with decrease in concentration than it would if it were directly proportional to the concentration, so that the observed decrease in rate is largely a concentration effect.

The absorption of the impure benzaldehyde used in the previous experiments was greater than that of any admixture with the chlorine compounds tested and was only exceeded when anthraquinone was added. The two pure samples (*a*) and (*b*) were from the same source, but (*b*) had been kept for 20 days after distillation. *m*-Chlorobenzoic acid appears to have a definite accelerating influence and it is rather remarkable that the effect is most evident during the second half hour of oxidation. The results for the other chlorine compounds indicate a slight retardation and it is therefore unlikely that these cause the activity of the impure benzaldehyde. Anthraquinone increases the reaction-rate very considerably as might be expected from its effect on pure toluene. In addition to those enumerated above, a few experiments were conducted in which benzoin and benzil were added to the benzaldehyde, but no acceleration was observed.

Effect of concentration upon reaction-velocity.

Numerous determinations of the rate of oxygen absorption in toluene solutions containing various proportions of benzaldehyde were made but results even more irregular than those shown in table I were obtained. A complete duplicate apparatus was erected in order that identical solutions could be exposed to light simultaneously. Even when this was done the results were not concordant and the source of the error could not be ascertained. As work on the subject has had to be discontinued, some typical results are given in table V illustrating the general nature of the effect of concentration. All solutions were made up by volume, i.e., a 25 per cent. solution is a mixture of 25 c.c. of benzaldehyde and 75 c.c. of toluene, the exposure being at a distance of 19 cm. from a pointolite lamp consuming 1·2 amps.

The first four experiments A–D formed part of an earlier series in which the absorption tube was immersed directly in a thermostat and consequently the conditions were not quite the same as later, when the duplicate indirectly heated apparatus was used; but it will be seen

TABLE V.

Absorption of oxygen by toluene solutions of benzaldehyde, 31°.

Time h.m.	Volume percentage of Benzaldehyde																	
	25	37·5	50	62·5	40		50					60			70	75	90	100
	A	B	C	D	14B	16B	4A	22B	28B	35B	21B	7A	14A	16A	7B	5A	5B	4B
20	3·5	13·4	31·0	42·5	15·7	15·1	30·5	30·0	32·3	33·9	30·1	37·1	37·8	34·6	40·2	34·0	32·7	31·0
40	4·2	13·0	28·0	37·5	17·3	17·4	34·2	31·5	33·4	38·3	34·6	37·8	38·1	35·6	41·2	29·7	29·5	33·1
1 0	4·8	12·9	26·0	30·5	17·7	16·1	34·7	32·4	32·3	39·7	40·0	38·7	36·5	34·4	41·0	27·7	29·1	31·3
1 20	5·0	11·7	22·0	26·0	18·9	17·4	31·9	29·7	35·8	38·6	43·9	38·1	36·9	33·4	40·0	29·3	29·7	32·4
1 40	4·8	11·3	20·2	24·3	17·1	15·8	29·4	26·8	34·4	39·0	44·9	34·9	34·0	33·5	38·4	30·0	29·6	31·5
2 0	4·5	10·7	18·0	22·0	15·8	16·1	24·5	21·1	29·7	38·3	38·4	33·5	30·4	30·4	36·6	28·6	29·8	32·1
2 20	4·1	9·4	17·2	19·0	14·2	13·4	20·1	17·8	25·3	33·1	44·8	31·2	29·1	27·6	34·7	27·4	29·2	30·6
2 40	3·8	9·2	16·1	17·3	13·1	11·9	18·2	17·0	19·6	25·7	32·4	27·8	26·8	25·8	33·3	27·5	28·5	29·9
3 0	3·7	8·6	15·1	16·5	13·1	10·2	17·3	15·4	15·9			26·3	25·1	23·3	29·4	27·9	27·7	28·2
3 20	3·7	8·0	13·6	16·3	10·2	9·9	16·3	15·0	15·2			24·7	20·9	21·7		26·6	28·1	29·0
3 40	3·6	8·0	13·3	16·4														
4 0	3·5	8·0	13·0	16·4														

that the initial rates for the 50 per cent. solutions are similar. In expts. B, C and D, the oxygen absorption rate fell steadily from the beginning; in several others it increased to a maximum at the end of about an hour and then diminished. After about two hours the rate appears to depend upon the concentration of the benzaldehyde, but the relation is barely more than qualitative. For example, in expt. C the oxygen absorbed in the interval 2 h. 40 m. to 3 h. was 15·1 c.c., and the benzaldehyde concentration at the end of the period was by calculation 40·8 per cent., agreeing approximately with the initial figures for expt. 16 B. The corresponding figures in expt. 4 A were however, 17·3 c.c. for a concentration of 38·7 per cent. and the rate was thus greater while the concentration was less. In expt. 14 A the oxygen absorption measured at the end of two hours was 30·4 c.c. and the concentration 50 per cent. agreeing with expt. 4 A, while in expt. 7 B after the same time, the absorption 36·6 c.c. and concentration 59 per cent. agree approximately with the initial figures for the latter concentration.

The general relation between velocity and concentration was complex. On drawing a curve connecting the average values it was found that the velocity increased slowly at first with increasing concentration; between 30 and 60 per cent. there was a very rapid rise to a maximum at about 70 per cent. and above this figure the rate fell nearly to the value for 50 per cent. solutions. If concentration were the main factor determining the rate, it would be expected that the figures for the 75 per cent. solution would have shown a considerable increase as the reaction progressed and the concentration was reduced to 70 per cent., but this was not the case. At the same time it is to be noted that the diminution in rate was much less for the highly concentrated solutions than for the 50 per cent. solutions and this is very probably due to the comparatively small change of velocity with concentration in the former case.

Expt. 21 B is given as an example of an abnormal reaction which proceeded very rapidly at an increasing rate until crystallisation set in after 2 h. 40 m. Crystal formation was usually observed when 310–320 c.c. of oxygen had been absorbed in the case of the 50 per cent. solutions, and 330–345 c.c. in the case of the more concentrated, the time varying from 3 to 5 hours.

Reactions in benzene solution.

As toluene itself is acted upon by light it appeared desirable to examine the reaction in a simpler solvent and some experiments were consequently conducted in benzene solution. Unfortunately it was not possible to complete the examination of solutions of different concentrations, but it was found that results with benzene were less irregular

than those with toluene. Table VI shows the oxygen absorption of 50 and 75 per cent. solutions compared with that of a sample of the same benzaldehyde with no solvent.

TABLE VI.

Absorption of oxygen by benzene solutions of benzaldehyde, 30·7°.

Time h. m.	Volume percentage of benzaldehyde		
	50	75	100
20	38·8	35·8	29·1
40	38·4	34·5	28·6
1 0	36·7	31·7	28·1
1 20	37·7 *	36·2 *	27·0
1 40	34·9	33·3	27·9 *
2 0	32·6	34·3	27·4
2 20	33·4 *	30·9	26·6

The values marked with a star were taken after the admission of fresh oxygen to the apparatus and cannot be regarded as reliable.

It will be seen that the course of the reaction differs in some respects from that in toluene solutions; the fall in rate of oxidation of the 50 per cent. solutions, is not so great when benzene is used as a solvent and no maximum rate is reached. The rate of oxidation of pure benzaldehyde is in both cases less than when a solvent is present, but with benzene the 50 per cent. solution is much more readily oxidised than the plain aldehyde, whereas in toluene the initial rates are similar. It is intended to examine more dilute solutions on a future occasion.

The effect of quinones.

Since anthraquinone has a marked accelerating influence upon the oxidation of toluene, as shown earlier in this paper, some experiments were conducted in which different quinones were added to benzaldehyde solutions. The volumes of oxygen at N.T.P. absorbed in successive periods of 20 minutes are shown in table VII.

In all the experiments except the first two, benzene was used as solvent. It will be observed in toluene solution anthraquinone acts as an accelerator whereas in benzene it does not, but the reaction

TABLE VII.

Absorption of oxygen in presence of quinones; 20 c.c. of 50 per cent. solution, 30·1°.

Expt. No.	Substance	Oxygen absorption								
30	0·1 g. anthraquinone in toluene ...	43·0	36·2	32·3	30·5	28·6	26·6	25·3	23·7	21·6
17	blank in toluene ...	29·8	27·3	25·4	22·4	20·0	17·2	15·7	14·2	13·3
72	0·1 g. anthraquinone in benzene ...	40·9	39·8	40·2*	37·2	35·2	38·4*	34·3	34·1	33·4
80	blank in benzene ...	41·2	39·7	39·2*	37·0	36·6	39·1*	34·5	32·5	33·1
90	0·076 g. benzoquinone	13·3	11·1	8·7	7·3	6·0	5·2	5·1	3·6	4·0
73	0·1 g. α-naphthaquinone	19·9	21·4	21·6	23·4	22·6	24·8*	23·5	26·0*	26·4
75	0·1 g. β-naphthaquinone	30·0	32·9	32·4	38·4*	36·5	34·7	34·5	33·3	31·7
82	0·03 g. phenanthraquinone	41·1	38·4	36·2	40·0*	38·1	36·3	38·9*	36·2	34·2
84	0·03 g. acenaphthaquinone ...	41·0	39·2	41·3*	39·0	...	39·9	35·8	32·8	...

in benzene alone is more rapid than in toluene even after adding anthraquinone. Benzoquinone is a strong inhibitor, probably owing to the absorption of the active light rays by its intensely yellow solution. The rapid diminution in the volume of oxygen absorbed is remarkable as there was no obvious colour change during the progress of the reaction. Similar effects were observed with more dilute solutions, the absorption for a solution containing 0·013 g. in 20 c.c. being 26·4 c.c. initially and 7·5 c.c. after 3 hours. The naphthaquinones were attacked, the solutions becoming paler as the reaction proceeded and this doubtless accounts for the initial retardation and subsequent increase in velocity. Phenanthraquinone and acenaphthaquinone solutions were completely bleached in about 15 minutes and hence their effect on the course of the reaction was negligible.

A series of experiments similar to the above was conducted using 10 c.c. of pure benzaldehyde, and almost identical results were obtained when allowance was made for the reduced rate of oxidation in absence of a solvent. Benzoquinone as before reduced the rate considerably, 0·017 g. giving rise to an initial oxygen absorption of 10·7 c.c. compared with 27·5 c.c. for benzaldehyde alone. The other quinones had little or no effect, the action of α-naphthaquinone being the most marked.

Before these experiments were made the rate of oxidation of different quantities of benzaldehyde in the same tube was measured and the rate found to be very nearly independent of the quantity. For example with 10, 20 and 30 c.c. of aldehyde the volumes of oxygen absorbed in 40 minutes were 53·3, 56·5 and 55·6 c.c., respectively, showing that the reaction takes place almost entirely at the surface of the tube which is always covered with a layer of liquid by splashing. If the liquid could be regarded as nearly saturated with oxygen and reaction took place throughout the mass, the larger volume would absorb most; if on the other hand the reaction rate were controlled by the speed at which the oxygen could dissolve, the small volume should absorb most, since in this case the largest surface is exposed to the gas.

The inhibiting action of phenolic compounds.

The antioxygenic action of pyrogallol and similar phenols has been extensively studied by other investigators and particularly by Moureu and Dufraisse (*loc. cit.*), but the latter authors allowed the reaction to proceed without agitation and in very feeble light so that it was not certain what relation their results would bear to those obtained under our experimental conditions. A series of experiments was therefore conducted using 10 c.c. of pure benzaldehyde in each case with small quantities of phenolic compounds. Table VIII shows

TABLE VIII.

Oxidation of benzaldehyde in presence of phenols, 30·5°.

No. 114	103	109	104	105	110	111	112	113	108
Blank	Quinol 4·3 mg.	Quinol 3·2 mg.	Quinol mono-methyl ether 4·1 mg.	Quinol dimethyl ether 4·5 mg.	Catechol 4·5 mg.	Resor-cinol 4·5 mg.	Pyrogal-lol 4·5 mg.	Pyrogal-lol 3·2 mg.	Pyrogal-lol tri-methyl ether 4·5 mg.
29·5	2·0	4·8	3·1	23·0	4·5	12·6	3·1	3·8	25·8
28·3	2·6	4·8	4·2	20·6	4·1	9·9	2·7	2·8	24·5
26·7	3·3	4·9	4·3	19·4	4·0	8·9	2·8	2·6	24·4
25·3	3·2	3·8	4·0	18·0	3·9	8·7	3·3	2·8	23·3
23·0	3·0	4·8	4·1	16·5	3·7	7·5	3·7	3·0	...
6·0[1]	2·8	5·3	4·3	16·3	4·7	7·9	3·8	3·5	23·8
...	4·0	5·3	4·6	16·9[2]	4·6	7·3	2·8	4·7	22·6
23·3	3·8	5·5	4·5	15·4	4·5	8·0	4·2	5·4	...
19·1	3·1	5·6	4·5	15·3	4·7	7·3	4·8	7·0	...
...	...	6·5	4·4	...	4·7	6·9	6·1	7·7	...
...	...	6·7	4·2	...	5·0	6·7	6·8	8·7	...
...	...	6·8	...	...	...	6·9	8·1	10·3	...
...	...	...	...	...	...	7·0	8·7	12·1	...
...	...	...	...	...	...	...	10·7	14·2	...
...	...	...	...	...	...	...	13·3	16·5	...

[1] No bubbling. [2] Oxygen added.

the results obtained, the figures representing c.c. of oxygen absorbed in successive periods of 20 minutes under standard conditions as to light intensity and rate of bubbling.

It is evident from these figures that all the compounds tested containing a free hydroxyl group were powerful inhibitors whereas the ethers had a much smaller effect. The antioxygenic action was moreover not permanent as shown by the values for quinol in expt. 109 and for pyrogallol, the increase in rate after three hours with the latter substance being very marked. In all cases of retardation the solutions become deep yellow, but attempts to isolate the coloured compound were unsuccessful. Absorption of light by this compound would not however account for the low rates of oxidation observed, as these were lower than those measured with no illumination (*cf.* next section).

Reaction in the dark.

At intervals during the measurements of the reaction velocity under the influence of light, comparative experiments were made in which the aldehyde was kept in the dark. Table IX shows some of the results, the volume of solution being 20 c.c. in the first four experiments and 10 c.c. in the others.

TABLE IX.

Absorption of oxygen in the dark; c.c. in 20 m., 29·5°.

No. 26	28	29	51	115	116	117	114
50 per cent. toluene solution			50 per cent benzene	No solvent		3·2 mg. pyrogallol	In light
6·1	6·8	8·4	33·4[1]	19·8	18·5	1·1	29·5
4·2	4·8	6·2	9·7	17·8	19·1	0·6	28·3
3·6	3·8	4·5	5·4	19·0	19·1	...	26·7
3·3	26·5[1]	3·4	4·6	18·5	18·7	...	35·3
3·3	6·5	3·1	...	18·8	17·9	0·8[2]	23·0
3·3	3·3	2·9	...	18·4	17·5	...	...
3·1	2·3	2·8	...	...	...	...	...
2·7	2·3	2·8	...	...	...	0·8[2]	...
2·7	2·3	2·7	...	...	...	...	...

[1] Exposed to light. [2] Total for 1 hour.

A striking feature of these figures is the rapidity of the oxidation of pure benzaldehyde in the dark when compared with that of its solutions. The retardation produced by adding 50 per cent. of toluene is

'en greater than in the case of the corresponding quantity of benzene ıd is evidently due to some specific action of the former solvent, as it ıs already been shown that there is a similar relation in the case of ıe light-catalysed reaction. The acceleration produced by light is ıuch greater with solvents, the total absorption being actually larger ı spite of the smaller absorption in the dark. The figures for the ›luene solutions show that the rate of absorption at the beginning of ıe reaction is greater than when it has proceeded for some time, and ı this respect they correspond with the observations of Moureu and ›ufraisse. The values in expts. 28 and 51 immediately after remo-ing the source of illumination indicate an after-effect. In the second ase measurements were taken at 10 minute intervals, and the actual alues were 5·9, 3·8, 2·9 and 2·5 c.c., showing that about half an hour ; required for the effect of the illumination to die down. The inhibi-ng action of pyrogallol is greater in the dark than in light, and a light tendency for the rate to increase with time was observed, 1·5 c.c. f oxygen being absorbed in an hour when the experiment had been n progress for four hours. A similar effect noticed by Moureu and)ufraisse was ascribed to distillation of the benzaldehyde in the eaction tube so that it was freed from the pyrogallol, but this expla-ation is not applicable in the present experiments.

The temperature-coefficient of the reaction.

A few measurements of the temperature-coefficients of the reaction n the dark and in light using pure benzaldehyde were made. Suffi-iently concordant results to give accurate values were difficult to ›btain, but the mean figures for absorption gave the following emperature-coefficients :—

In the dark	20·5–30·5°	1·32	30·5–40·5°	1·10
In light	25·0–35·0°	1·09	30·5–40·5°	1·18

Reiff (*loc. cit.*) obtained the value 1·3 for the range 15–25° and ›ointed out that this is characteristic of a diffusion reaction.

Discussion of results.

The results so far obtained are insufficient to justify any generalisations regarding the nature of the reactions, but serve to indicate the direction in which further work should proceed. While not directly at variance with any theories previously advanced, they show that numerous factors must be taken into consideration and that the entire mechanism of the reaction may vary according to the conditions. In the case of benzaldehyde, solvents have a marked effect, benzene and toluene retarding the reaction in the dark, but accelerating it in light

if not present in too large a proportion. The accelerating influence of benzene is greater than that of toluene, while an experiment with *p*-xylene showed that this substance inhibits the reaction almost completely. There thus appear to be two opposing factors introduced into the photochemical reaction by the addition of solvents, one increasing the velocity by a dilution effect and the other diminishing it in different degrees depending upon the oxidisability of the solvent itself. The relation between reaction velocity and concentration is obscure and requires investigation in a greater variety of solvents, while minor effects such as the small rise in reaction rate soon after the commencement of the reaction in the light, and the rapid initial fall in the dark, should not be neglected.

The greatest difficulty encountered has been the inconsistency of results obtained under apparently identical conditions. Although some improvement might be effected by a device for exposing the liquid to the action of light and oxygen in the form of a thin film moving at a constant rate, the main variations seem to depend upon minute quantities of impurity in the benzaldehyde itself, the nature of which it has not been possible to determine. The addition of various chlorine compounds has been found to have little or no effect, while anthraquinone is the only substance observed to have a definite accelerating influence; even this is only in toluene solutions. The well-known antioxygenic action of phenolic compounds has been confirmed, but it has been shown that the retarding effect in light is far less than in the dark, pyrogallol in particular soon tending to become less active, presumably owing to its gradual decomposition.

The similarity of the temperature coefficients in light and in the dark, and the independence of the rate and the volume of the liquid, indicate that the reaction in both cases takes place mainly at the surface, Reiff having clearly established this fact for the reaction in the dark. The after-effect noticed upon removing the source of light shows the presence of an intermediate compound, but this must be either a different body to the one formed in the dark or its equilibrium concentration must be greatly diminished when not exposed to light. The different behaviour of pyrogallol in light and in darkness suggests that the former view is more probable.

The oxidation of toluene differs considerably from that of benzaldehyde. It takes place very slowly or not at all in the absence of a catalyst and appears to be a volume reaction, the rate probably depending upon the extent of the water-toluene interface. Although formed in small quantities, benzaldehyde is not the intermediate product which determines the rate of the reaction, the active substance being hydrogen peroxide, an or organic peroxide which is readily

even greater than in the case of the corresponding quantity of benzene and is evidently due to some specific action of the former solvent, as it has already been shown that there is a similar relation in the case of the light-catalysed reaction. The acceleration produced by light is much greater with solvents, the total absorption being actually larger in spite of the smaller absorption in the dark. The figures for the toluene solutions show that the rate of absorption at the beginning of the reaction is greater than when it has proceeded for some time, and in this respect they correspond with the observations of Moureu and Dufraisse. The values in expts. 28 and 51 immediately after removing the source of illumination indicate an after-effect. In the second case measurements were taken at 10 minute intervals, and the actual values were 5·9, 3·8, 2·9 and 2·5 c.c., showing that about half an hour is required for the effect of the illumination to die down. The inhibiting action of pyrogallol is greater in the dark than in light, and a slight tendency for the rate to increase with time was observed; 1·5 c.c. of oxygen being absorbed in an hour when the experiment had been in progress for four hours. A similar effect noticed by Moureu and Dufraisse was ascribed to distillation of the benzaldehyde in the reaction tube so that it was freed from the pyrogallol, but this explanation is not applicable in the present experiments.

The temperature-coefficient of the reaction.

A few measurements of the temperature-coefficients of the reaction in the dark and in light using pure benzaldehyde were made. Sufficiently concordant results to give accurate values were difficult to obtain, but the mean figures for absorption gave the following temperature-coefficients :—

In the dark	20·5–30·5°	1·32	30·5–40·5°	1·10
In light	25·0–35·0°	1·09	30·5–40·5°	1·18

Reiff (*loc. cit.*) obtained the value 1·3 for the range 15–25° and pointed out that this is characteristic of a diffusion reaction.

Discussion of results.

The results so far obtained are insufficient to justify any generalisations regarding the nature of the reactions, but serve to indicate the direction in which further work should proceed. While not directly at variance with any theories previously advanced, they show that numerous factors must be taken into consideration and that the entire mechanism of the reaction may vary according to the conditions. In the case of benzaldehyde, solvents have a marked effect, benzene and toluene retarding the reaction in the dark, but accelerating it in light

if not present in too large a proportion. The accelerating influence of benzene is greater than that of toluene, while an experiment with *p*-xylene showed that this substance inhibits the reaction almost completely. There thus appear to be two opposing factors introduced into the photochemical reaction by the addition of solvents, one increasing the velocity by a dilution effect and the other diminishing it in different degrees depending upon the oxidisability of the solvent itself. The relation between reaction velocity and concentration is obscure and requires investigation in a greater variety of solvents, while minor effects such as the small rise in reaction rate soon after the commencement of the reaction in the light, and the rapid initial fall in the dark, should not be neglected.

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decomposed, but is not destroyed by pyrogallol, this compound actually increasing the rate of reaction instead of reducing it as in the case of benzaldehyde.

In conclusion we wish to express our best thanks to Mr. S. K. Kulkarni Jatkar for much assistance during the course of the experiments.

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[Accepted, 24-3-31.]

CANTHARIDIN FROM MYLABRIS PUSTULATA Fb., INDIA.

By B. H. Iyer and P. C. Guha.

Cantharidin is a colourless, crystalline substance much used as a vesicant, and is contained in more than a dozen medicinal preparations for internal and external use. Owing to its high blistering power internal administration requires great caution, as it irritates the alimentary canal and causes marked vasoconstriction ; a cantharidin-content of 1 in 100,000 is the prescribed internal dose. Cantharidin is used in tuberculosis, lupus, cystitis, incontinence of urine and many other diseases. It is used externally in the form of plasters and tincture more than internally, and is also used in very minute doses as a hair-tonic, cantharidin hair-oil being an example.

Cantharidin is extracted from a type of insect commonly known as blister beetles, formerly used as such, or as an oily extract for blistering. R. Hughes Buller's observation in this connection is worth mentioning : "In India these beetles are used in a somewhat similar manner. In Baluchistan, for instance, the 'Gojak' beetle (closely allied to *Mylabris pustulata*) is soaked in milk and the fluid poured on the bald spots to cure mange." (From *Indian Insect Pests*, by H. Maxwell-Lefroy).

The blister beetles belong to the order *Coleoptera*, the two important species of this order which yield cantharidin being *Cantharis vesicatoria*, or Spanish Fly and *Mylabris pustulata*. The former are widely distributed in southern Europe and are quite different in appearance from *Mylabris pustulata*, being longer and having green upper wings. In Europe they are the chief source of cantharidin, but owing to the large variety of insects found in the tropics, the exact nomenclature and species of the family used for this work could not be identified with certainty from the available literature. Specimens were therefore sent to the Government Entomolgist, Coimbatore, and afterwards to the Imperial Bureau of Entomology, London, for confirmation, when the insects proved to be *Mylabris pustulata* Fb., India.

At present, the dried insects, or extracted cantharidin preparations are imported at high cost, and an ample supply of blister beetles being available locally, the opportunity was taken to ascertain the cantharidin-content of these. Inquiry into the possibility of rearing the insects as a source of cantharidin has shown that their life-history is not yet

sufficiently well known to offer a prospect of success. The Government Entomologist, Coimbatore, informs us that the triangulin, or first stage grub, feeds upon grasshopper eggs, and this requirement suggests that it would be more profitable to collect the adult beetles at those periods when thousands infest the blossoms and tender shoots of numerous plants. Moreover, their removal for cantharidin-extraction would help to diminish their destructive activity, which in some districts is a serious menace to crops and gardens.

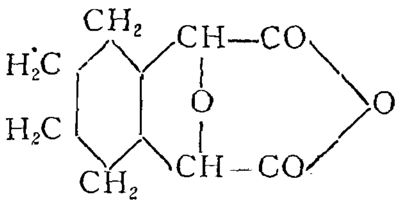
CONSTITUTION OF CANTHARIDIN, $C_{10}H_{12}O_4$.

The establishment of a constitution for cantharidin required more than thirty years (from 1892 to 1923) of laborious work, by J. Gadamer (*Arch. Pharm.*, 1914, **252**, 609 and 636; 1917, **255**, 290 and 315; 1920, **258**, 171), Walter Rudolph (*Arch. Pharm.*, 1916, **254**, 423) and Samuel Coffey (*Rec. trav. chim.*, 1923, **42**, 387 and 1026). J. Gadamer in his first communication (*loc. cit.*) has very ably reviewed the numerous results of previous workers and hence it is only necessary to give here an account of the work of Gadamer and his successors. Danckworth (*Arch. Pharm.*, 1914, **252**, 632), one of Gadamer's collaborators has shown that cantharidin dissolved in acetone neutralised more caustic potash than is required for one carboxyl group and prepared a dibasic brucine salt which could not be resolved into active forms. Thus he concluded that cantharidin is the anhydride of a dibasic acid (cantharidic acid), and definitely fixed the state of three among the four oxygen atoms in cantharidin. Gadamer in his third memoir (*Arch. Pharm.*, 1914, **252**, 636) described the action of hydrobromic acid on cantharidin, obtaining hydrobromocantharic acid and cantharidin dibromide, $C_{10}H_{12}Br_2O_3$, analogous to the diiodide of Piccard (*Ber.*, 1886, **19**, 1404). From these experiments he considered the fourth atom of oxygen to be present in an ether group, the action of halogen acids being

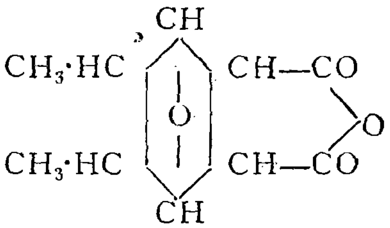
$$\underbrace{R{-}O{-}R'}\ + HI \rightarrow \underbrace{R{-}OH\quad R'I}\ + HI \rightarrow \underbrace{RI\quad R'I} + H_2O$$

The optical inactivity of cantharidin, and the fact that active hydrobromocantharic and cantharic acids always give inactive cantharidin, led to the belief that cantharidin does not contain an asymmetric carbon atom. On treatment with hydriodic acid cantharidin yields cantharic acid, $C_{10}H_{12}O_4$, and a diiodide, $C_{10}H_{12}I_2O_3$. When this derivative is heated with a solution of caustic potash in a sealed tube, pure cantharene (dihydro-*o*-xylene) is produced, yielding *o*-toluic and phthalic acids on oxidation with nitric acid. When sodium cantharidate is distilled with soda-lime, cantharene, xylene and high boiling ketones distil (J. Piccard, *Ber.*, 1879, **12**, 577).

Based on these facts, Gadamer (*Arch. Pharm.*, 1914, **252**, 624) suggested the following three formulæ for cantharidin:



I



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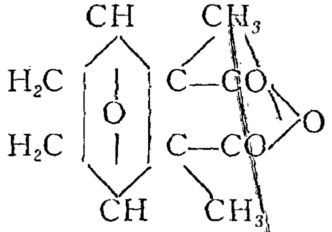
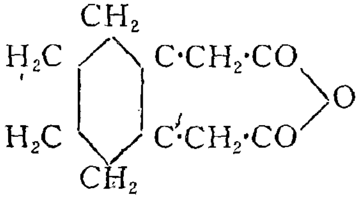


III

without pronouncing definitely in favour of any one, each having been found applicable to some of the reactions. At this juncture Samuel Coffey (*Rec. trav. chim.*, 1923, **42**, 387 and 1026) reviewed the past literature and made the first attack on the synthetic side.

When dibromocantharidin is reduced, deoxycantharidin arises (Rudolph, *Arch. Pharm.*, 1916, **254**, 423), and according to Gadamer's formulæ might have a constitution represented by one of the formulæ,



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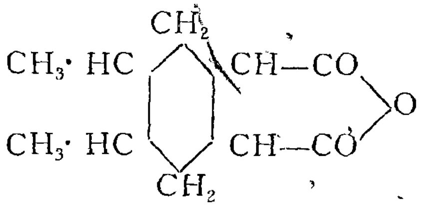
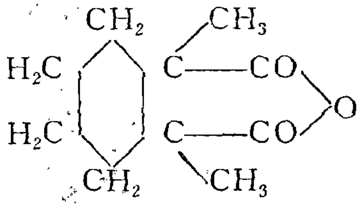


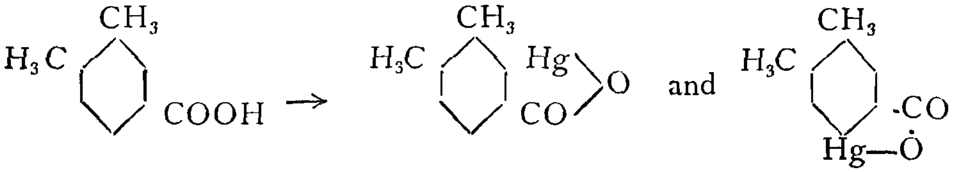
V



VI

These three formulæ correspond to the names (IV) *cis-cyclo*hexane-1:2-diacetic anhydride, (V) *cis*-4:5-dimethyl*cyclo*hexane-1:2-dicarboxylic anhydride and (VI) *cis*-1:2-dimethyl*cyclo*hexane-1:2-dicarboxylic anhydride.

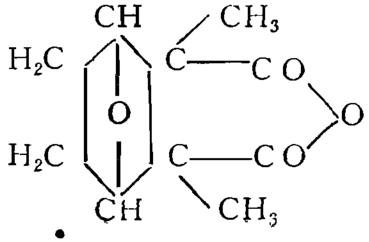
Coffey prepared *cis-cyclo*hexane-1:2-diacetic acid and its anhydride by catalytic reduction of *o*-phenylenediacetic acid, and the products being different from deoxycantharidic acid and deoxycantharidin obtained from cantharidin, formula (I) was rejected. *cis*-Hexahydro-4:5-dimethylphthalic anhydride was prepared from *p*-xylic acid, itself obtained by oxidising ψ-cumene with nitric acid (Fittig and Laubinger, *Annalen*, 1869, **151**, 275; Perkin and Bentley, *J. C. S.*, 1897, **71**, 166). On mercuration of *p*-xylic acid (VII) by Presci's method, Coffey produced two *o*-hydroxymercuri-*p*-xylic anhydrides (VIII) and (IX),



VII VIII IX

which could not be separated: hence the mixture was sulphonated, the alkali salts of the sulphonated products being easily separated by fractional crystallisation. The potassium salt of the corresponding sulpho-*p*-xylic acid on fusion with sodium formate, yielded dimethylphthalic acid which in turn was reduced by hydrogen in presence of platinum. Along with *cis*-hexahydro-4:5-dimethylphthalic acid, a small quantity of the corresponding phthalide was produced. This acid and its anhydride were not identical with the deoxycantharidic acid and deoxycantharidin, respectively; hence the second of Gadamer's three formulæ (II) was also rejected.

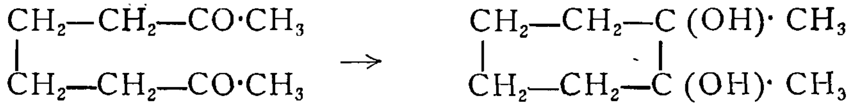
So by a process of elimination the third formula, to which Gadamer himself was more inclined, was confirmed, the accepted constitution for cantharidin thus being



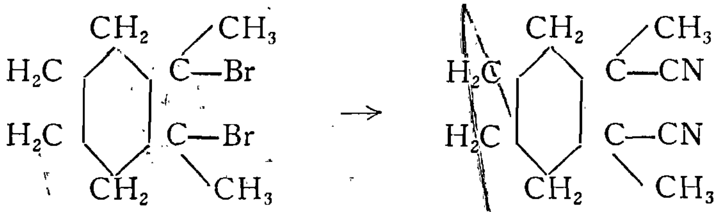
III

ATTEMPTS TO SYNTHESISE DEOXYCANTHARIDIN.

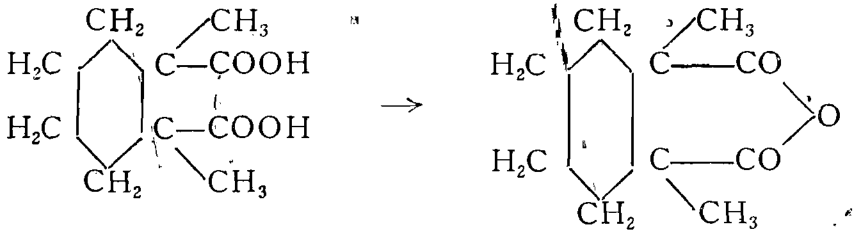
A synthesis of either deoxycantharidin or cantharidin itself will be the only definite confirmation of the above. It was proposed to start with ωώ-diacetylbutane (X) and subject it to pinacone condensation when product (XI) is to be expected. This on bromination should give 1:2-dibromo-1:2-dimethyl-*cyclo*hexane (XII), yielding with potassium cyanide the dinitrile (XIII). When this is treated with acid the dicarboxylic acid (XIV) i. e., deoxycantharidic acid is to be expected, giving deoxycantharidin (VI) by dehydration.



X XI



XII XIII



XIV VI

Owing to the extreme difficulty of preparing diacetylbutane in sufficient quantity this plan had to be abandoned. However, the dibromo-compound (XII) was obtained from *o*-methyl*cyclo*hexanone (Hans Meerwein, *Annalen*, 1918, **417**, 268), although for want of sufficient experimental details much difficulty was encountered. Meerwein states that the dibromide (XII) cannot be obtained in pure condition, as it immediately darkens. Although the bromination was conducted in absence of light the product would not remain pure for a reasonable period, and further difficulties were encountered on treatment with potassium cyanide. This reaction, although tried more than a dozen times under varying conditions, has always given a tarry mass from which nothing could be separated, even in absence of any hydrolytic agent. Further experiments in these lines are in progress.

EXPERIMENTAL.

Collection and Preservation of the Beetles.

These beetles are found in large flights between August and November; being herbivorous they are very great pests. Their habit is very sluggish during the early morning with increasing activity as the sun rises; hence the collection was made between 6 and 11 a.m.

Mylabris pustulata possess good power of flight. A yellow-coloured, bad smelling oil is exuded from the leg-joints, and the dissolved cantharidin besides causing blisters, perhaps renders them unpalatable to enemies such as birds. Beetles used in this work are black, with three orange-red bands across the elytra, and varied from 0·5 to 1·0 inch in length with average weight of 0·5 g. They emit a very acrid, nauseating odour. When alive their appearance is bright, but the orange bands lose their brilliance and the insects become dull when dead, and having very little moisture they do not decay when kept unpreserved. Collections were made from fields cultivated for cereals or vegetables in the neighbourhood of Yeshwantapur, Bangalore City, during January, February, March and April, by hand-picking. In Bangalore these insects appear throughout the year, but during the months of July, August and September they are found in greater abundance.

The insects were killed by chloroform, dried in the sun for two or three days and preserved whole or powdered. In this process, a power-driven mill was found more efficient than the mortar and pestle, which leave the wing-sheaths uncrushed. No preservatives were used excepting a few naphthalene balls. The beetles when dried lose half their original weight.

Extraction of Cantharidin.

Before extraction, a qualitative test for cantharidin was made according to the method of L. David (*Pharm. Z.*, 1927, **72**, 56). Three beetles were crushed in a porcelain mortar with 25 c.c. of chloroform and 5 to 10 c.c. of aqueous ammonia; after evaporation of the filtered liquid, 6 to 8 drops of fuming nitric acid were added, the residue dried and warmed with a few drops of concentrated hydrochloric acid containing vanillin (1 g. in 100 c.c.) when an orange-red coloration immediately developed indicating cantharidin.

Several methods of extraction involving different organic solvents are described in the literature. Cantharidin is markedly soluble in hot alcohol, chloroform, ethyl acetate and boiling benzene; it occurs as a salt (potassium cantharidate) as well as in the free state, so the first

step to complete extraction is to decompose the salt, either by alkali or acid. The general methods of extraction can be grouped in two categories. By the first, total cantharidin (i.e., after decomposing the salt) along with the fat in which cantharidin is very soluble is extracted with an organic solvent, either chloroform or ethyl acetate, the solvent distilled, and the fat removed either by carbon disulphide or light petroleum. In the second method, due to Puran Singh (Imp. Forest Research Inst. Reprint, 1907, 16; *J.C.S.*, A., ii, 1907, 994), an inorganic extracting medium is employed. The salt is first decomposed by alkali and the cantharidin extracted by means of a dilute solution of alum which when concentrated throws out cantharidin along with the basic salt of aluminium; this is removed by hydrochloric acid and the cantharidin crystallised from alcohol.

The method of E. Dieterich (*J. Pharm.*, 1893 (5), **27**, 375; *J.C.S.*, A., i, 1893, 600) was followed with some modifications in the working details.

Experiment I (*for free cantharidin only*).—37 Grams of sun-dried and powdered cantharides were extracted in a glass soxhlet with chloroform, which was distilled after running for 24 hours; and the residue treated with cold carbon bisulphide to remove all fatty material. The crude cantharidin was crystallised from absolute alcohol and melted at 218°, not depressed by admixture with a genuine sample from Merck. The yield was 0·5 g., amounting to 1·35 per cent. of the dried beetles.

Experiment II (*for total cantharidin*).—100 Grams of dry powdered cantharides were treated with 150 g. of ethyl acetate and 2 g. of concentrated sulphuric acid; after two days 4 g. of barium carbonate was added and the mixture extracted in a glass soxhlet for 48 hours with ethyl acetate. The residue after distilling the solvent was macerated several times with cold light petroleum (b. p. 40–60°), the crude cantharidin being treated with a small quantity of hot alcohol and allowed to cool. By this process the small quantity of a resinous product giving a green fluorescence in alcoholic solution was removed. 46 Grams of pure cantharidin were obtained from 2 kilos of dried beetles, the yield being 2·3 per cent. From the two experiments described above, it can be seen that 0·95 per cent. of the substance exists in the form of potassium salt. Cantharidin being slowly volatile, insects kept for long periods gave smaller yields. A sample of Spanish Fly (from Europe) supplied by the Bengal Chemical and Pharmaceutical Works, Calcutta, gave practically no yield, because they might have been very old. Moreover Spanish Fly contains much less cantharidin than *Mylabris pustulata*.

The volatility of this material suggests extracting cantharidin before drying the beetles in the sun, to preclude loss. Hence the crushed insects after being subjected to sulphuric acid and barium carbonate treatment successively were extracted with chloroform. The whole process was very unpleasant and the yield was very poor, evidently due to the extraction not being efficient.

The dried insects yielded 12 to 12·5 per cent. of fat the examination of which is described in the next paper by Iyer and Ramaswami Ayyar. There it has been shown that the fatty oil still retains one more per cent. of cantharidin which could not be isolated by the usual methods, the total cantharidin content thus reaching 3·3 per cent. Although it is obtained as a by-product the fat is of very great value as it could be used as such for external applications of cantharidin.

SOME REACTIONS OF CANTHARIDIN.

In attempts to elucidate the constitution of cantharidin, various derivatives and reactions have already been studied. In some cases, especially with phenylhydrazine, several compounds were obtained. As the constitution was not known at that time, no definite explanation has been offered.

The action of 4-phenylthiosemicarbazide, urea, guanidine hydrochloride and hydrazine hydrate on cantharidin has now been tried, but no reaction could be observed in the first three cases, either in alcoholic solution or by heating a mixture of the two substances at a high temperature.

Cantharidin Hydrazide.—To 5 g. of cantharidin mixed with a few c.c. of absolute alcohol was added under ice cooling an alcoholic solution containing 3·75 g. (twice the theoretical quantity) of hydrazine hydrate. There did not appear to be any change until room temperature was reached when the reaction started, and on keeping overnight the whole quantity of the solid went in solution. After boiling this for an hour on a water bath, alcohol was distilled and the solid crystallised from alcohol, melting at 118°. The hydrazide is very soluble in water, ether, acetone, and is also soluble in alcohol, hot petroleum (b.p. 50-60°), benzene, dilute acetic and hydrochloric acids. Yield, 5·5 g., i.e., 80 per cent. of theory (Found: C, 56·83; H, 5·72; N, 13·72. $C_{10}H_{14}O_3N_2$ requires C, 57·14; H, 6·67; N, 13·33 per cent).

Benzylidene derivative.—One gram of the hydrazide dissolved in water was well shaken with 0·7 g. of benzaldehyde and then heated on a water bath for half an hour. On slow cooling crystals separated,

and after recrystallisation from 98 per cent. alcohol, melted at 158°. (Found: N, 9·78. $C_{17}H_{18}O_3N_2$ requires N, 9·39 per cent.).

The preparation of ωω′-diacetylbutane was tried according to Marshall and Perkin (*J.C.S.*, 1890, **57**, 24) their method being the only one known; but although the experiment was repeated over a dozen times no workable quantity could be obtained. Consequently experiments were conducted on the electrolysis of potassium laevulinate according to Hans Hofer (*Ber.*, 1900, **33**, 650), who has not given any experimental detail as to the electrodes; several experiments with nickel and carbon electrodes were tried, but were unsuccessful.

Preparation of 1 : 2–*Dimethyl*cyclo*hexene.*

Following Meerwein (*Annalen*, 1914, **405**, 145), the scanty experimental details allowed only a poor yield to be obtained in the first few experiments; improvement resulted from the following modifications. 100 Grams of freshly fused and well powdered zinc chloride were heated, in a round bottomed flask fitted with a long air condenser protected by a calcium chloride tube, to 120° on an oil bath. On cooling to 90°, 50 c.c. of 1 : 2-dimethyl*cyclo*hexanol were added and the temperature raised to 170-180° for 10 to 15 minutes. Further 15 g. of zinc chloride was added and the product rapidly distilled, dried over calcium chloride and redistilled, when 32 g. of the pure hydrocarbon (b.p. 132–134°) was obtained. Heating the dimethyl*cyclo*hexanol for a longer time invariably gave an inferior yield.

CONCLUSION.

The maximum yield of cantharidin hitherto recorded is 1·9 per cent. from Chinese beetles, but the foregoing experiments having shown a yield of 2·3 per cent. it is evidently quite feasible to produce it in India. Moreover, the cost of laboratory-production, representing labour of collection and value of materials without deduction for recovery of ethyl acetate, amounts roughly to 6 annas per gram, which compares very favourably with the cost of imported cantharidin at Rs. 2–3–0 per gram.

Our thanks are due to M.R.Ry. Rao Sahib Y. Ramachandra Rau Garu, M.A., F.E.S., Government Entomologist, Coimbatore, for kindly identifying the beetle.

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THE FATTY ACIDS FROM OIL OF CANTHARIS (MYLABRIS PUSTULATA Fb., INDIA).

By B. H. Iyer and P. Ramaswami Ayyar.

In the previous communication entitled 'Cantharidin from *Mylabris Pustulata* Fb., India' it has been shown that a fatty oil could be obtained by boiling the ethyl acetate extract of the insects with petroleum ether. The cantharis oil so prepared does not appear to have been examined chemically before.

A casual reference to a cantharidin oil from *Lytta Vesicatoria* and to another from the Spanish Fly occurs in the literature (Lewkowitsch, Oils and Fats, 1922, Vol. II, p. 489; Beilstein, I, 455; Gossamann, *Annalen*, 1853, **86**, 317; 1854, **89**, 123); but no analytical data have been given for the oils or their component acids. It was therefore of interest to make a preliminary chemical examination of the oil with the quantity at our disposal.

The work described below shows that the fatty oil prepared as above retains much cantharidin in solution and that the separation of the pure fat, free from cantharidin, was attended with considerable difficulty. The fatty acids present in the oil appear to be a mixture of palmitic, stearic, Δ-9: 10-oleic and an isomeric oleic acid with the double bond probably in the 6:7 position. The unsaponifiable matter, forming 5 per cent. of the oil is a liquid mixture from which a small portion of a waxy solid (m.p. 60-65°) could be obtained. On account of the small quantity available it was not examined further.

EXPERIMENTAL.

The ethyl acetate extract from two kilos of the dried insects was boiled in about twenty lots with petroleum (b.p. 40-60°) for several hours, under reflux, and the solution filtered hot each time. From the combined solution the petroleum was distilled, leaving 250 g. of crude fat, a yield of 12.5 per cent. of the dried insects. The fat was yellowish-brown and had an unpleasant odour.

ACID VALUE.

The crude fat on titration with standard caustic soda solution in the usual manner, showed the abnormally high acid value of 150. This high acidity led us to suspect the presence in solution of much cantharidin in the fat, and this was confirmed by a very strong orange-red coloration with a hydrochloric acid solution of vanillin, characteristic of cantharidin.

NEUTRAL FAT.

The crude fat (20 g.) was neutralised with dilute caustic potash solution and the mixture extracted with ether to remove the neutral fat. The ethereal solution was separated with great difficulty from the aqueous emulsion and yielded after removal of the solvent nearly 5 g. of a pale yellow fat melting at 30° to clear yellow liquid. The fatty oil had a specific gravity of 0·9106 at 30°/24° and its refractive index was 1·4714 at 30°. The preparation of much neutral oil from the crude fat was thus very tedious and the analytical data for the crude fat would only give misleading indications of its composition owing to admixture with much cantharidin. No further analysis of the oil was therefore made. Hence the crude fat was converted into the free fatty acids and these were examined further.

PREPARATION OF UNSAPONIFIABLE MATTER.

In a typical hydrolysis of the crude fat for the preparation of the mixed acids 100 g. of the fat was boiled for several hours under reflux with a solution of 40 g. of caustic soda in about 300 c.c. of 90 per cent. alcohol. The alcohol was then distilled and the resulting soap dried at 100°, powdered and extracted with ether in a Soxhlet apparatus. The ethereal solution after washing, drying and evaporation of the solvent gave 5·0 g. of unsaponifiable matter. After two or three crystallisations from a mixture of alcohol and ether a small amount of a white, waxy solid separated melting at 60–65°, the remainder being an oil which yielded no crystalline derivatives. On account of the small quantity available the substance was not examined further.

THE MIXED ACIDS.

The ether extracted soap was decomposed with boiling dilute (1 : 1) hydrochloric acid and the liberated fatty acids recovered in a pure condition in the usual manner; yield, 80 g.

REMOVAL OF VOLATILE ACIDS.

As the analytical data of the mixed acids (see Table I) show the possible presence of appreciable quantities of lower fatty acids, the former (80 g.) were subjected to steam-distillation during eight hours when the distillate was no longer acidic. The steam distillate was neutralised with baryta water, evaporated to small bulk and decomposed with hydrochloric acid, when an appreciable amount of cantharidin crystallised, and was identified by a mixed melting point with the pure substance. The residual mixed acids weighed 73 g. and still gave a positive vanillin test for cantharidin.

SEPARATION INTO SATURATED AND UNSATURATED ACIDS.

The steamed mixed acids were further separated into the solid and liquid acids, by the well-known lead salt alcohol method (*J. Ind. Eng. Chem.*, 1921, **13**, 806). In a trial separation of the unsteamed mixed acids the solid acids formed 30·2 per cent. and the liquid 69·8 per cent. In the steamed acids the respective proportions were 33·3 and 66·7 per cent. The analytical data of the above acids are summarised in Table I.

TABLE I.

	Unsteamed		Steamed	
	Equivalent	Iodine value	Equivalent	Iodine value
Mixed acids ...	220·0	42·6	251·2	43·4
Solid acids ...	258·8	2·1	285·0	6·3
Liquid acids ...	231·9	54·4	248·3	61·2

THE SOLID ACIDS.

As the quantity available was not quite sufficient for separation by fractional distillation of the methyl esters, a preliminary examination was made as follows. The solid acids (26 g.) prepared from the steamed mixed acids were dissolved in warm petroleum (40–60° b.p.) and the solution allowed to crystallise. The first crop of 4 g. was pure stearic acid (m. p. 68–70°), unchanged on mixing with pure stearic acid. Equivalent, 286, found: C, 76·2; H, 12·4 per cent. $C_{18}H_{36}O_2$ requires, equivalent, 284, C, 76·0; H, 12·7 per cent. The residual acids recovered from the mother liquor were crystallised from 90 per cent. alcohol giving 3 g. of a crystalline acid melting at 56–57° and having the equivalent 295. This corresponds to a mixture of stearic acid (60 per cent.) with a higher acid, probably arachidic (40 per cent.); but these could not be isolated in a pure condition. The alcoholic mother liquor from the second crop gave on evaporation to dryness 19 g. of a solid acid which had the equivalent 283·8 and an iodine value 8·6. Only stearic acid (equivalent 284) could be isolated from this solid acid which is therefore assumed to be a mixture of 17·1 g. of stearic and 1·9 g. of oleic acid. Thus 26 g. of the saturated acids appear to consist of 23·1 g. of stearic, 1·9 g. of oleic and 1 g. of (probably) arachidic acid. The solid acids as a whole did not give any reaction for cantharidin.

THE LIQUID ACIDS.

The low iodine value (61·2) of these showed the absence of acids with higher unsaturation than oleic; and the low equivalent (248·3) is mainly due to admixture with cantharidin, the presence of which was confirmed by the vanillin test. A preliminary examination of its composition was therefore attempted by the method of oxidation with alkaline permanganate solution, whereby dihydroxystearic acid formed from oleic acid could be easily separated from the other constituents which were further analysed as follows.

Oxidation.—The steamed liquid acids (35·5 g.) were neutralised exactly with alcoholic potash and the solution evaporated to dryness. The residual potassium salts dissolved in 3,500 c.c. of water were treated at 0° gradually with 700 c.c. of an ice-cold 1.5 per cent. solution of potassium permanganate with vigorous mechanical stirring during one hour. After standing for one more hour at 0°, the precipitated manganese dioxide was completely dissolved by sulphur dioxide, and a white crystalline precipitate of the hydroxy-acid resulted.

TABLE II.

<table>
<tr><td colspan="7">Liquid Acids (35·5 g.) oxidised by $KMnO_4$.</td></tr>
<tr><td colspan="3">(I)
Water insoluble (12 g.) treated with petroleum and filtered.</td><td colspan="4">(II)
Water soluble (12 g.) extracted by ether, treated with petroleum and filtered.</td></tr>
<tr><td rowspan="3">(IV)
Petroleum insoluble 2 g.</td><td colspan="2">(III)
Petroleum soluble 9 g. Prepared Pb-salts in alcohol and filtered.</td><td rowspan="3">(IX)
Petroleum insoluble 5 g.</td><td colspan="3">Petroleum soluble. Prepared Pb-salts in alcohol and filtered.</td></tr>
<tr><td rowspan="2">(V)
Acid from insoluble Pb-salts 6·7g.</td><td rowspan="2">(VI)
Acid from soluble Pb-salts 2·3 g.</td><td rowspan="2">(VI)
Acids from soluble Pb-salts 1 g.</td><td colspan="2">Insoluble Pb-salts treated with ether and filtered.</td></tr>
<tr><td>(VII)
Acid from soluble Pb-salts 1 g.</td><td>(VIII)
Acid from insoluble Pb-salts 1 g.</td></tr>
</table>

Excess of sulphur dioxide was then expelled from the solution by heating and the precipitated acids (I) consisting of hydroxy-acids and

any unchanged material were filtered off. The aqueous filtrate saturated with salt was extracted with ether several times to recover the water soluble as well as oxidation products mechanically carried over (II). The further treatment of (I) consisted in removing by means of petroleum unchanged oleic and any other higher monobasic acids (III, V and VI) from the dihydroxystearic acid (IV), while the further treatment of (II) consisted again in removing by means of petroleum the monobasic acid products of oxidation (VII and VIII) from the dibasic acids present including cantharidin (IX). Further separation of the monobasic acid constituents by means of their lead salts in alcohol or ether resulted in the removal of unchanged oleic acid (VI) from the saturated monobasic acids (V and VII) present. The whole scheme of the above separations is outlined in Table II.

Analysis of the Oxidation Products.

Fraction (IV) which formed the crude dihydroxystearic acids (2 g.) gave on crystallisation from alcohol 0·4 g. of a white crystalline substance melting at 130–131° (unchanged on mixing with pure dihydroxystearic acid. Found: C, 69·2; H, 10·9, $C_{18}H_{36}O_4$ requires C, 68·4; H, 11·4 per cent. Equivalent, 320; calculated, 316).

The alcoholic mother liquor from the above yields what appears to be an isomeric dihydroxystearic acid, m.p. 120–122°.

Fraction (V) was practically pure palmitic acid melting at 60–62° (unchanged on mixing with pure acid. Equivalent, 254; calculated, 256).

Fraction (VI) is oleic acid having equivalent, 285; n_D^{25} 1·4585; iodine value, 53·3 after one hour. Oleic acid has equivalent 282; n_D^{25}, 1·4600; iodine value, 90·0.) The acid does not absorb bromine as readily as ordinary oleic acid.

Fraction (VII) is lauric acid, identified by its equivalent, 197·4 instead of 200, and melting point.

Fractions (VIII) and (IX) appear to be all cantharidin.

From the above analysis, allowing for loss due to the complete oxidation of a portion of the acids, the liquid acids (35·5 g.) appear to consist of palmitic acid (6·7 g.) and oleic acid (24·2 g.) from the original iodine value, and the remaining amount of 4·6 g. represents admixed cantharidin.

The occurrence of a small quantity of lauric acid among the oxidation products, the formation of a low-melting dihydroxystearic

acid and the isolation of a small quantity of an oleic acid whose analytical values differ from those of the ordinary oleic acid, point to the probable existence in the liquid acids of an isomeric oleic acid with double bond in the 6 : 7 position, namely,

$$CH_3(CH_2)_{10}\cdot CH : CH\cdot(CH_2)_4\cdot CO_2H.$$

The occurrence of palmitic acid in the liquid acids is probably due to the incomplete precipitation of its lead salt from the original mixed acids. The case of lauric acid, however, seems to be different as no evidence of its presence was found in the original mixed acids, when they were steam distilled.

CONCLUSION.

The fatty acid mixture obtained by the hydrolysis of the crude fatty oil obtained from *Mylabris pustulata* Fb., India, has been shown to have the following approximate composition :—

Unsaponifiable matter	5·0	per cent.
Palmitic acid	11·0	,,
Stearic acid	27·0	,,
Oleic acid and isomeride	46·0	,,
Arachidic acid	1·0	,,
Dissolved cantharidin	10·0	,,

It is rather remarkable that the fatty oil tenaciously retains so large an amount of cantharidin in solution, the usual methods of extraction failing to recover this from the fatty oil. A new method must be evolved for complete extraction of cantharidin.

Our thanks are due to Dr. P. C. Guha for his kind interest in this investigation.

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I.—AMYLASE FROM RICE.

By Dattatreya Vishnu Karmarkar and Vinayak Narayan Patwardhan.

The present communication forms a part of a series on amylases from cereals, of which some have already been published (*Journal Indian Inst. Sci.*, 1928, **11A**, 121; 1929, **12A**, 185; 1930, **13A**, 31, 159). The rice-malt amylase possesses properties similar to those of other cereal amylases examined in this laboratory. Ungerminated paddy does not contain any active enzyme.

EXPERIMENTAL.

Preparation of the enzyme.—Paddy requires steeping for four days at room temperature (24–26°) before it can be spread out to germinate. After four days, when the plumules are about half an inch high, the seedlings are collected, sun-dried and treated as usual for preparation of dry malt.

Since rice contains only a small quantity of water-soluble proteins, it was very difficult to obtain an active precipitate of the enzyme by the method of Ling and Baker (*J. C. S.*, 1895, **67**, 702). An active preparation was, however, obtained on extracting the malt powder with a 0·6 per cent. solution of sodium bicarbonate and precipitating with 95 per cent. alcohol. It was also observed that if malted rice is allowed to stand overnight in contact with water and 95 per cent. alcohol then added to the extract, the precipitate is as active as that obtained from the alkaline extract of the malt. The reaction of the water extract after twenty-four hours was P_H 4·8, that of the fresh extract being P_H 5.9. Active enzyme was also obtained adjusting the reaction of the fresh extract to P_H 4·8 by adding dilute acid followed by precipitation with alcohol. The enzymes obtained by the three methods mentioned above were found to be equally active.

HYDROLYSIS OF STARCH BY RICE AMYLASE.

(*a*) *Saccharification.*—To 100 c.c. of 2 per cent. starch solution, 20 c.c. enzyme solution (0·02 per cent.) was added with 1 c.c. of toluene. The mixture was maintained at 38° and sugar estimated at

ıtervals. The results are compared with the corresponding figures ɔr wheat-malt in Table I.

TABLE I.

Time in hours	Maltose in terms of c.c. N/20 thiosulphate	
	Rice	Wheat
½	1·8	8·2
1	3·8	17·1
2	10·1	23·2
4	15·0	24·6
8	21·8	24·8

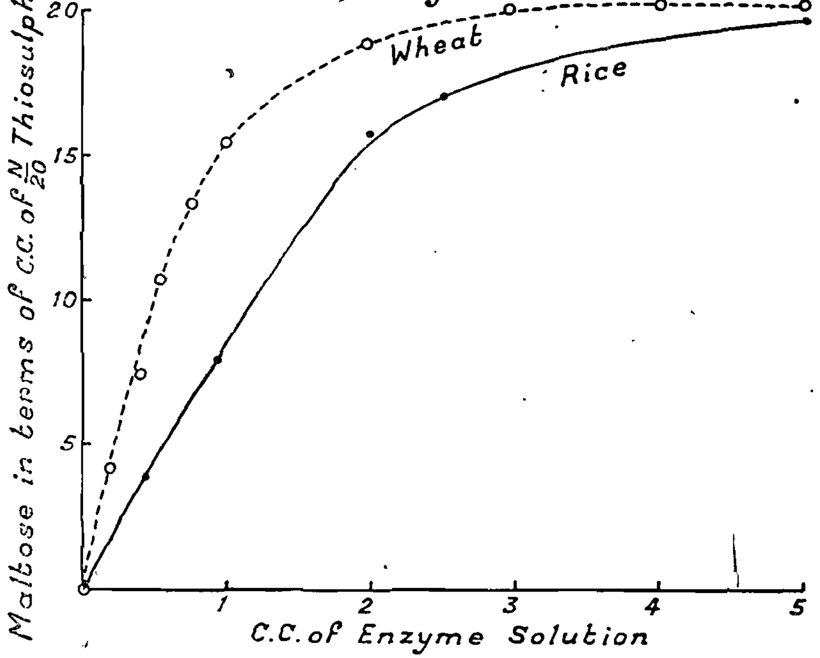
It may be noted that in the earlier stages the wheat-malt amylase ; about five times as active as the one from rice-malt; in estimating quefaction of potato-starch the enzymes were therefore taken in the atio 1 : 5.

(*b*) *Liquefaction.*—Potato-starch paste (25 c.c. of 2 per cent.) ʼas mixed with 25 c.c. of rice amylase (0·1 per cent.) at 30° and 10 .c. of the mixture transferred to a viscometer maintained at 30°. The eadings taken at intervals were compared with those in a similar xperiment with a 0·02 per cent. solution of wheat-malt amylase Table II).

TABLE II.

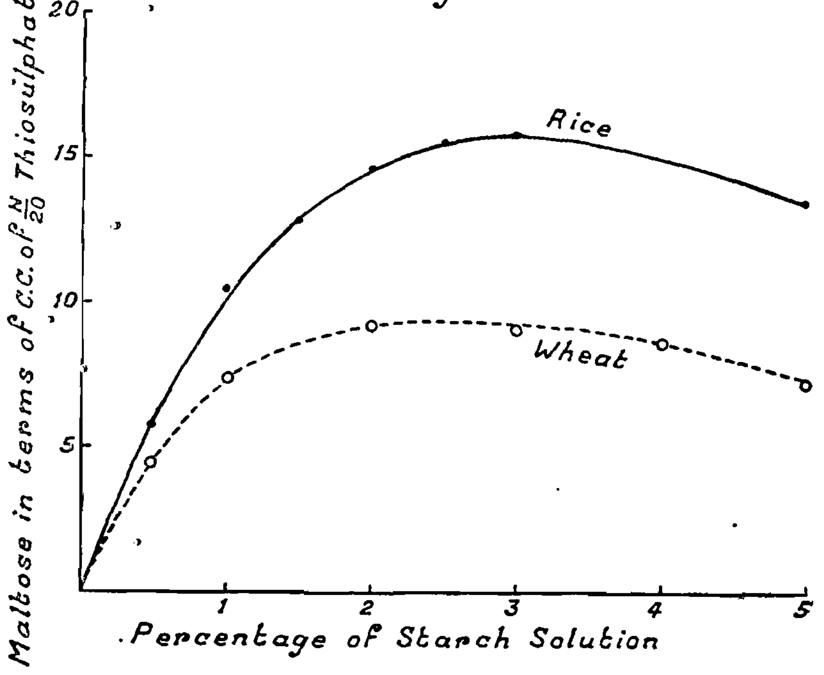
Time in minutes	*t* in seconds	
	Rice	Wheat
0	201	176
15	117	112
30	107	109
60	107	109
90	106	108
120	105	107

Fig. I



Maltose in terms of C.C. of $\frac{N}{20}$ Thiosulphate
20
15
10
5
Wheat
Rice
1
2
3
4
5
C.C. of Enzyme Solution

Fig. II



Maltose in terms of C.C. of $\frac{N}{20}$ Thiosulphate
20
15
10
5
Rice
Wheat
1
2
3
4
5
Percentage of Starch Solution

The observations show that rice amylase liquefies starch to the same extent as wheat amylase if the rates of saccharification are adjusted so as to be equal in both the cases.

Effect of temperature on rice amylase.—To each of a series of flasks containing 45 c.c. of starch solution (2 per cent.) 5 c.c. of malt extract (8 per cent.) was added after bringing the former to the desired temperature. The reaction was allowed to proceed for 30 minutes and then arrested by 5 drops of 30 per cent. sodium hydroxide. The mixtures were cooled and their respective sugar contents determined with the necessary correction for sugar content of malt.

TABLE III.

Temperature...	25	35	40	45	50	55	60	65	75
Maltose (c.c. of N/20 thiosulphate) ...	9·1	10·6	11·6	14·5	15·1	15·5	15·8	14·1	3·7

Effect of reaction.—To each of a series of flasks containing 40 c.c. of starch solution (2 per cent.) and 10 c.c. McIlvaine's buffer solution adjusted to a particular reaction, 10 c.c. of enzyme solution (0·10 per cent.) were added and the mixtures incubated at 38° for 1 hour.

TABLE IV.

PH ...	3·8	4·0	4·2	4·4	4·6	4·8	5·0	5·2	5·	5·6	5·8	6·0	6·4
Maltose (c.c. of N/20 thiosulphate) ...	8·8	9·6	10·2	11·61	(1·7	11·4	11·2	11·0	· ·	9·5	8·6	8·0	7·2

Effect of concentration :—(*a*) *Substrate.*—To seven flasks each containing 50 c.c. of starch solution of a particular strength 10 c.c. of rice amylase solution (0·08 per cent.) was added, and sugar estimated after incubation for one hour at 38°. The results are presented in Fig. I.

(*b*) *Enzyme.*—A series of nine flasks each containing 50 c.c. of starch solution (2 per cent.) and a particular quantity of water were arranged. The total volume was made up to 60 c.c. in each case by adding the required volume of enzyme solution (0·40 per cent.). The flasks were incubated for 1 hour at 38° and sugar determined as usual; the results are given in Fig. II.

Effect of electrolytes and amino-acids on the activity of rice amylase.—The experiments were conducted with electrolyte-free enzymes under controlled conditions of temperature and reaction, with and without addition of buffer solutions. The details of the experiments were the same as those described in the previous communications by Patwardhan and his co-workers (*loc. cit.*).

The electrolytes tested were the chlorides of potassium, sodium and calcium, and the sulphate, oxalate, malate and tartrate of potassium, in 0·0025, 0·005 and 0·010 molar concentrations. In no case could any effect on the activity of amylase be observed as the result of the adding of any of the electrolytes. The amino-acids, glycine, leucine, *l*-alanine, aspartic acid and hippuric acid in concentrations similar to those described above, also led to negative results.

Effect of prolonged dialysis.—The enzyme loses most of its activity on dialysis in collodion bags (6 per cent., 2 layers) prolonged for about fifteen days. The results of the above and other similar experiments are discussed in the following paper.

SUMMARY.

1. An active preparation of rice amylase can be obtained by extracting the malt (*a*) with dilute (0·6 per cent.) alkali or (*b*) after adjusting the reaction to P_H 4·8 followed by precipitation with 95 per cent. alcohol and subsequent dehydration with absolute alcohol and ether.

2. The activity of rice amylase is less than one quarter that of the corresponding wheat enzyme.

3. The optimum temperature of the rice-malt enzyme is 60° and optimum reaction, P_H 4·6. The enzyme does not respond to addition of electrolytes or amino-acids, and on continued dialysis it loses its activity in fifteen days.

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II.—DIALYSIS OF SOME CEREAL, AMYLASES.

By Vinayak Narayan Patwardhan and Dattatreya Vishnu Karmarkar.

Investigations on the properties of cereal amylases carried out in this laboratory have already shown that these enzymes lose activity when subjected to continued dialysis. Attempts to ascertain the cause of this phenomenon have led to the conclusion that it is due to the progressive destruction of the enzymes themselves owing to certain hitherto obscure causes. The present investigation has shown that the deterioration is not due to the relatively high room temperature since it has been found that aqueous solutions of similar enzyme preparations retained more than 50–75 per cent. of their original activity even after more than two months in glass containers, while on prolonged dialysis nearly all their activity was lost within about three weeks.

Sherman and his co-workers (*J. Amer. Chem. Soc.*, 1919, **41**, 1867; 1921, **43**, 2461) suggested that amylase loses its activity owing to the hydrolysis of enzyme protein by proteases accompanying the enzyme. The present investigation has however shown that such a hypothesis is untenable. Supposing that the enzyme proteins were hydrolysed during dialysis, they would pass out of the collodion bag and thus bring about a steady decrease in the total nitrogen of the enzyme solution. Figures given in the experimental portion of the text show that with the progress of dialysis the nitrogen content reached a minimum and remained subsequently constant, while the activity continued to diminish to the very end. A direct proof of the untenability of Sherman's hypothesis was also obtained by testing the action of proteolytic enzymes on amylase solutions. The experimental details of such an experiment have been given in a previous paper (*J. Indian Inst. Sci.*, 1930, **13A**, 159), where it was shown that pepsin inactivated wheat amylase in aqueous solution, the rate depending upon the concentration of pepsin; papain and trypsin were without effect. All three proteolytic enzymes were allowed to act on amylase solutions at neutral reaction, since it was observed that loss of activity during dialysis took place in the neighbourhood of P_H 6·7. Similar experiments carried out with amylases from rice, barley, cholam (*Sorghum vulgare*), maize, ragi (*Eleusine coracana*) and bajri (*Pennisetum typhoideum*) failed to show any inactivation due to the presence of proteolytic enzymes. These results do not support Sherman's hypothesis regarding the destruction of the enzyme nor do they supply evidence for the proteid nature of amylase as advocated by him.

Possibility of enzyme adsorption by collodion membranes was also tested by trying to elute the enzyme from the membrane, but no trace of adsorbed enzyme could be obtained by elution.

The dialyses were conducted carefully and sterile, toluenated water was used throughout. The bacterial count actually decreased with the progress of dialysis, and the possibility of bacterial decomposition of the dialysing enzyme was thus eliminated.

As a preliminary to experiments on dialysis, it was found that membranes made with two coats of 6 per cent. collodion did not allow the enzyme to pass through them. The same type of membranes were also used for electro-dialysis. Membranes prepared according to Frick and Kaja (*Ber.*, 1924, **57**, 310) were found to be unsatisfactory.

Some of the previous workers found electro-dialysis to be a rapid means of purifying amylases. Thus Narayanamurti and Norris (*J. Indian Inst. Sci.*, 1928, **12A**, 134) purified cholam malt amylase by electro-dialysing it through parchment membrane. Similar attempts to purify other amylases by electro-dialysis led, however, to almost complete destruction of the enzymes concerned. The details of our experiments were the same as those described by Narayanamurti and Norris except that rigorously tested collodion membranes were used in place of parchment ones. Of the seven amylases subjected to electro-dialysis, only wheat amylase retained its activity, all others being rendered inactive.

Rice amylase was first dialysed in the electro-dialyser at room temperature without passing the current. After three days, the terminals were connected to the main (110 D.C.) with a resistance of 2800 ohms in series. It was observed that the inactivation began at once, and at the end of four hours the activity was reduced by fifty per cent. It is possible that the destruction of the enzyme was due to the high potential between the terminals, because the enzyme could not have diffused out, or adsorbed on the membrane, or been precipitated with protein: it is however difficult to reconcile the above observation with those of Fricke and Kaja on commercial amylase (*loc. cit.*) Narayanamurti and Norris on cholam amylase (*loc. cit.*) and our own on wheat amylase.

In the present investigation, the activities of the enzyme solutions were compared on the volume basis rather than that of solid content as is usually done. Thus if a given solution of enzyme shows an activity x remaining unchanged when a large amount of inert solids have passed out of the dialysing membrane, the activity can be said to have remained unaffected, even though according to the solid content estimations it must have increased. During dialysis a stage is reached when solid content remains constant while the activity continues to

diminish till it can be said to have been practically lost. This would mean that the solids undergo changes leading to the destruction of the hydrolytic properties of the enzymes and thereby converting them into inert substances. The mechanism of that process can be understood only by a physico-chemical study of the enzyme in solution. Investigation of such a nature has been undertaken in this laboratory and the results will be published shortly.

EXPERIMENTAL.

The methods of preparing malts and enzymes are the same as those described in the previous communications (*loc. cit.*).

I.—*Estimation of total and soluble nitrogen content of cereal malts and their enzymes.*

Since it is believed that proteins act as carriers of the enzyme and since there is considerable fall in the nitrogen content during dialysis, it was considered that a study of total and soluble nitrogen of these enzymes might be useful.

A weighed quantity of malt (3–4 g.) was digested with 100 c.c. of cold water during 1 hour, filtered and made up to 150 c.c. Concentrated sulphuric acid (5 c.c.) was added and the contents evaporated nearly to dryness on the water bath. Nitrogen was then determined by the kjeldahl method.

TABLE I.

Malts				Precipitated enzymes	
	Percentage		Days required for inactivation	Percentage	
Malt	Total N	Soluble N		Total N	Soluble N
Wheat ...	2·43	0·58	15	...	5·35
Barley ...	1·76	0·28	35(*ca.*)	...	5·85
Rice ...	1·32	0·17	15	6·85	2·30
Maize ...	1·77	0·26	3–4	...	4·34
Ragi ...	1·33	0·27	9	...	2·78
Bajri ...	1·74	0·43	13	...	5·08
Jawari ...	1·53	0·21	...	...	1·56

II.—*Prolonged dialysis of cereal amylases.*

Portions of 20 c.c. of the malt extract (30 per cent.) were dialysed in a collodion bag, several being in one dialyser. At known intervals bags were removed, the contents filtered and made up to 100 c.c. Nitrogen, solid content and activity were determined. The results are given in tables II, III and IV.

TABLE II.

Rice Amylase.

Days	P_H	Solids per cent.	Nitrogen in c.c. of 0·1071 N alkali	Maltose in c.c. of N/20 thiosulphate
0	5·9	2·13	6·75	15·00
1	6·4	0·62	1·50	14·00
3	6·7	0·04	1·00	13·90
6	6·5	0·05	0·50	6·80
8	6·7	0·05	0·50	6·10
15	6·7	0·05	0·50	1·70
Control after 15 days ...	5·9	2·12	6·75	15·0

TABLE III.

Bajri Amylase.

Days	P_H	Solids per cent.	Nitrogen in c.c. of 0·1071 N alkali.	Maltose in c.c. of N/20 thiosulphate
0	5·7	4·20	15·50	14·70
2	6·2	0·49	3·50	11·70
5	6·5	0·10	1·50	4·20
7	6·7	0·08	1·00	3·30
10	6·9	0·08	1·00	1·80
13	6·9	0·08	1·00	0·70
Control after 13 days ...	5·7	4·20	15·00	13·50

TABLE IV.

Barley Amylase.

Days	P_H	Solids per cent.	Nitrogen in c.c. of 0·1071 N alkali	Maltose in c.c. of N/20 thiosulphate
0	5·6	5·12	16·75	16·70
3	6·3	0·20	3·50	15·00
6	6·7	0·13	1·75	15·60
8	6·9	0·12	1·0	14·80
11	6·9	0·08	1·0	14·50
14	6·9	0·08	1·0	14·20
35	...	...	...	0·30
Control after 35 days ...	...	5·11	16·50	17·30

III.—*Effect of ageing on dialysed amylase.*

Portions of 20 c.c. of the malt extract (30 per cent.) of wheat and rice were dialysed during three days when the enzymes were practically free from electrolytes and reducing sugars.. The solutions were preserved for over two months with added toluene, and their activities determined at intervals.

TABLE V.

Date	Maltose in c.c. of N/20 thiosulphate	
	Wheat	Rice
14—11—1930	15·4	12·1
3—12—1930	15·3	12·2
10—12—1930	15·5	11·9
18—12—1930	13·4	9·5
23— 1—1931	11·9	6·3

IV. *Testing for possible adsorption of enzyme by the collodion membrane.*

Four collodion bags, each containing 20 c.c. of the amylase from wheat enzyme solution (0·1 per cent.) were preserved for dialysis as

usual. After 15 days when the amylase was almost inactivated, the bags were removed, contents separated and the bags washed with small quantities of distilled water. Two bags were then cut to pieces and eluted with 20 c.c. of the elutive agent (100 c.c. consisting of 57 c.c. of 1 per cent. $(NH_4)_2HPO_4$, 3 c.c. of N ammonia and 40 c.c. of 87 per cent. glycerol) at P_H 7·8. Two other bags were similarly treated with another elutive agent at P_H 4·6 (100 c.c. consisting of 40 c.c. or McIlvaine's buffer of P_H 4·6 and 60 c.c. of 40 per cent. glycerol). After four hours, 10 c.c. were taken from each flask and dialysed to remove glycerol. The activity was then tested against starch, and in no case could any activity be detected showing that the enzyme was not adsorbed on the membrane used for dialysis.

V. *Electro-dialysis of amylases.*

(*a*) An electro-dialyser very similar to that of Fricke and Kaja (*loc. cit.*) was used. Laboratory supply (110 volts, D.C.) with a resistance of 2,000 ohms in series was used as a source of current.

A stock solution of 500 c.c. of malt extract (15 per cent.) was prepared, 20 c.c. being made up to 50 c.c. and kept as control; 400 c.c. was electro-dialysed till the conductivity approached that of distilled water. The solution was then removed, made up to 1000 c.c. and filtered, the solids being inert. The activity of the filtrate was then determined and the results are given in Table VI.

TABLE VI.

Malt	Control		Electro-dialysis		
	P_H	Maltose in c.c. of N/20 thiosulphate	Days	P_H of middle cell	Maltose in c.c. of N/20 thiosulphate
Rice ...	5·8	15·0	3	4·5	1·3
Wheat ...	5·9	14·8	2	4·6	14·7
Barley ...	5·6	16·1	3	4·4	0·9
Maize ...	5·6	8·4	3	4·6	1·2
Ragi ...	5·6	12·2	3	4·4	2·0
Bajri ...	5·7	12·9	3	4·5	0·2
Cholam ...	5·6	6·8	3	4·7	0·3

(*b*) Rice-malt extract was dialysed in the electro-dialyser without the passing current, which was then switched on and the enzyme electro-dialysed, in one case for four hours and in the other for six hours. Their respective activities are given in Table VII.

TABLE VII.

Activity before electro-dialysis	Activity after electro-dialysis		
Maltose in c.c. of N/20 thiosulphate	Electro-dialysis hours	P_H	Maltose in c.c. of N/20 thiosulphate
14·2	6	4·5	6·4
12·9	4	4·5	6·5

SUMMARY.

1. All the cereal amylases hitherto investigated have been found to lose their activity on prolonged dialysis.

2. Electrolyte-free amylase in aqueous solution retained more than 50 per cent. of its original activity even after two months.

3. On electro-dialysis through collodion membranes, all cereal amylases excepting that from wheat lost their activity.

4. Probable causes of destruction of the enzymes have been investigated.

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CONTRIBUTIONS TO THE STUDY OF SPIKE-DISEASE OF SANDAL (*SANTALUM ALBUM*, LINN.).

Part XII. Ecology of Sandal.

By M. Sreenivasaya and S. Rangaswami.

The study of sandal in its natural habitat has a fundamental interest from the standpoint of the parasitic nature, and also in respect of the economic efficiency, of the tree, which is determined by (1) vigorous growth resulting in high volume increment, (2) profuse seeding, ensuring an extensive regeneration of species, (3) intensive heartwood formation rich in the valued essential oil and (4) resistance to disease and insect attack. In the case of the parasitic sandal these factors are mainly controlled through its associated host-plants; the richest soil which may be provided, by itself does not favour the growth of sandal, the nutrients of the soil being made available to sandal largely through suitable host-plants which are haustorised by sandal. Pot-culture experiments have established the interesting fact that sandal has a preference in general for host-plants of the leguminous type, which seem to favour its rapid and vigorous growth.

The frequency and quantity of seeding is influenced by the kind and number of hosts with which sandal happens to be associated. Pot-cultured sandals deprived entirely of their host-plants seed only once a year, usually in the month of November, and yield seeds with practically *no* germinating power, while sandals freely fed by hosts *normally* seed twice a year. It has been found that sandal-seeds collected from various localities vary widely in their germinating capacity; and a systematic study of the vitality of sandal-seeds in relation to the floristic composition of the host-groups should reveal the nature of the host-plants which impart to sandal the quality of bearing fruits in profusion and of high fertility.

It is believed that heartwood formation is influenced by conditions reputed to disfavour vigorous vegetative growth, but reliable and accurate data are not available in support of this theory. Girth for girth, a sandal struggling in a rocky soil and in association with xerophytic plants, has higher proportion of the oil-bearing heartwood than one thriving luxuriantly on a rich soil, enjoying good rainfall and nourished by vigorous hosts. But the girths of two such sandals are

not comparable as regards age, since in the one case growth is exceedingly slow, while in the latter girth-increment is rapid. It is not known whether heartwood formation is at all a function of age, but there is no doubt that it is influenced by the nature of associated host-plants and by the moisture conditions of the soil. Further, the percentage of essential oil in the heartwood has been found to vary with the ecological aspect of the locality. Heartwood from Javadis for example has the reputation of possessing a high oil-content, and in consequence fetches high prices.

The resistance of sandals to insect attack has a two-fold significance. Primarily, sandal is liable to attack by several insect types, tending to restrict growth, and secondly, certain types by virtue of their feeding habits and biological relationships are inimical to sandal as vectors of disease. It is well known that, generally, the internal physiological condition of the plant determines its predisposition to insect attack and this condition in the case of the parasitic sandal is influenced by its host-plants. The variety and quantity of insect fauna infesting a sandal will therefore depend upon the floristic composition of the host-groups nourishing it.

Analysis of pot-cultured sandal nourished by known hosts, has shown that the host has a considerable influence on the composition of the sandal sap, thereby rendering the plant more or less open to insect attack. By a judicious choice of host-plants, it should be possible to control the composition of the sap in such a way as to render it distasteful or repulsive to those classes of insects reputed to be vectors of disease. This would represent only a static type of immunity, and the sandal would succumb to disease if the infective agency attacked the plant through some means other than the insect.

Perfect and unqualified immunity is one which enables the sandal to combat the infection after its internal invasion, through some inherent mechanism. The parasitic sandal is possibly dependent on its host-plant for this dynamic defensive function as well, and herein lies perhaps the secret of a successful and rational elucidation of spike-disease, which has been causing heavy damage to some of the richest sandal-bearing tracts of Mysore, Coorg and Madras.

It has been observed that under natural conditions the sandal-bearing areas vary widely in their capacity to function in respect of the four main factors indicated above, as they are largely dependent upon the floristic composition of the areas. Table I shows that sandal attains higher girths and survives longer in certain areas than in others. The yield of heartwood also varies with different areas.

TABLE I.

Growth and heartwood formation in some sandal-bearing areas.

	Locality	Mean annual girth increment, inches	Percentage of heartwood	Average exploitable size, inches
Madras	Chitteries ...	0·75	4·77	60
	Nilgiris ...	0·60	12·60	36
	Javadis ...	0·66	4·00	...
	North Salem ...	0·25	3·70	...
Mysore	Heggad Devankote ...	0·40	9·09	31·5
	Chamarajnagar ...	0·40	5·10	36·0
	Hunsur ...	0·36	8·60	36·0

If natural regeneration of species can be taken as a true measure of reproductive capacity the self-sowing of sandal in different areas will be different, and this is found to be the case. The resistance of sandals to disease has also been found to vary widely with different areas. While some areas have remained immune to the disease in spite of their close proximity to infected areas, there are others which have succumbed to the infection in spite of their comparative isolation from sources of infection. It has been found that the rate of spread of spike-disease is higher in certain areas while in others the spread is very slow indeed, as can be seen from Table II.

TABLE II.

Locality	No. of Spiked Sandals		Observation period, months
	At start	At close	
Tholuvubetta ...	4	62	24
Mahadeswarangudi ...	1	13	33
Jawalgiri (Bungalow) ...	6	109	10
Jawalgiri (Cairn 53) ...	3	12	12

An ecological survey of some typical sandal-bearing areas was undertaken to determine the extent to which host-plants influence the parasitic sandal in performing its useful functions. Special attention has been given to the possible effect suspected of hosts in rendering sandal either susceptible or resistant to spike-disease. The

sylvicultural and other climatic characteristics prevailing in the surveyed areas are tabulated below.

TABLE III.

Locality	Elevation, feet	Soil	Type of forest	Average annual rainfall, inches	Temperature, mean	Humidity
Devarabetta Healthy	3,144	Disintegrated gneiss with rocky subsoil	Open scrub	31·7	21·5	76
Devarabetta Spiked	3,150	Do.	Do.	31·7	21·5	76
Nognoor Healthy	3,000	Do.	Do.	35·0	21·5	76
Nognoor Spiked	2,900	Do.	Do.	35·0	21·5	76
Uduparani	3,050	Do. mixed with boulders	Do.	37·0	21·5	76
Aiyur	2,950	Do. with sandy loam	Do.	35·0	21·5	76
Jawalgiri	3,100	Disintegrated gneiss	Do.	35·0	21·5	75

Methods of survey.—Three different methods were employed in the surveys, and were selected according to the nature of the area to be surveyed. Reconnoitring survey was conducted in the Uduparani area, since the severe slopes and rugged undulations do not lend themselves to a survey by other methods. The plot-strip method was employed for Devarabetta areas, which comprise continuous and practically uniform growth of sandal. The group system of surveying was found most suitable for the Nognoor area, where the sandal growth is not continuous, but occurs generally in patches. The manurial plots at Aiyur and Jawalgiri have been surveyed by the plot method.

The frequency and intensity of occurrence of each species of tree and shrub has been determined in all areas. The occurrence or the absence of a particular species has been broadly classified under the following heads :—

(1) Significant absence or diminished occurrence (Table IV) ;
(2) exclusive presence or significant preponderance (Table V) ;
(3) dead or coppiced stumps and lantana (Table VI).

DISCUSSION.

The differences found between healthy and diseased areas are very striking and suggestive. Diseased areas are characterised by a complete absence or diminished occurrence of certain types of flora

like *Barleria buxifolia*, *Cippedessa fruticosa*, *Dodonea viscosa*, *Breynia rhamnoides*, etc., while the preponderance of certain species of plants in healthy areas lends significant support to the theory that disease resistance in the sandal plant is controlled by particular types of host-plant. The high percentage of coppiced and dead stumps of host-plants, presumably exploited for fuel, found in diseased areas is very significant and supports the observation made in the laboratory that infected sandal succumbs to the disease more rapidly in absence of a host-plant.

The frequent association of abundant lantana with diseased areas led to the belief that it had some bearing on disease incidence. The surveys have confirmed this, which is placed on a quantitative basis by Table V; but the exact value of this plant must remain a matter for future investigation. The rapid spread of lantana in a given forest area is invariably the result of either indiscriminate exploitation of the stock or frequent and severe fires, both of which result in opening out the forest and favour the growth of lantana. In other words, the sandal stock in the area suffers a change from deep-rooted and sturdy host-trees to surface-feeding bushes of doubtful host-value like lantana, *Acalypa Indica*, and others.

The following table connects the diseased areas so far surveyed with a significant absence or diminished occurrence of the following species of plants.

TABLE IV.

Name of Plant	Relative concentration of species in areas	
	Healthy	Spiked
Asparagus racemosus	71	17
Atylosia albicans	243	26
Barleria buxifolia	30	0
Breynia rhamnoides	204	4
Bridelia retusa	68	3
Canthium didymum	82	41
Cipadessa fruticosa	24	0
Cudrania Javanensis	15	0
Dioscorea oppositifolia	164	49
Dodonea viscosa	603	253
Ehretia buxifolia	70	31
Erythroxylon monogynum	124	68
Holarrhena antidysenterica	18	0
Memecylon edule	229	26
Pittosporum floribundum	104	0
Scutia Indica	116	43
Toddalia aculeata	148	52

The following table connects the diseased areas so far surveyed with an exclusive presence or a significant preponderance of the following species of plants.

TABLE V.

Name of plant	Relative concentration of species in areas	
	Healthy	Spiked
Acacia pennata	5	153
Acacia sundra	0	27
Atalantia monophylla	3	135
Clausena willdenovii	22	136
Lantana camara	284	4747
Limonia acidissima	0	6
Phyllanthus polyphyllus	14	375
Pterolobium Indicum	0	18
Strobilanthus kunthianus	78	169
Triumfetta rhomboidea	6	71

TABLE VI.

Locality	Dead trees		Lantana	
	Healthy	Spiked	Healthy	Spiked
Devarabetta	32	143	355	4787
Nognoor	41	134	284	4747

One of the most striking observations is the constant association of agricultural operations with the primary site of the first disease attack. In the case of every one of the diseased areas so far investigated there has not been a single exception. Artificial interference with virgin areas by cutting down the host-plants associated with sandal has been responsible for rendering it susceptible to disease, new outbreaks of which have always taken place in areas close to agricultural fields or colonies; but we are still far from tracing the ultimate origin of the infective principle.

SUMMARY.

1. The extent to which sandal is dependent upon host-plants for performing its useful physiological functions is indicated.

2. An ecological survey of the sandal-bearing areas in North Salem has been conducted chiefly to determine the influence of associated hosts on the susceptibility or resistance of sandal plants to spike-disease. These surveys are being extended to other types of forest in Coorg and Madras.

In conclusion, our thanks are due to Mr. J. E. M. Mitchell, District Forest Officer, North Salem, for the kind interest he has taken and for the facilities given us in conducting the survey.

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STUDIES IN ELECTRO-CULTURE.' PART I.
INTRODUCTION.

By K. Venkata Giri, T. J. Mirchandani and V. Subrahmanyan.

Investigations carried out in different parts of the world during the past half-century leave little doubt regarding the possibility of increasing crop-production by treating plants with electricity under favourable conditions. The factors affecting the efficacy of the treatment are however, so many, and the conditions so difficult to control, that although many workers have obtained almost phenomenal increases in crop-yields, several have reported to the contrary, often the same authors working under apparently identical conditions. The information so far obtained is not sufficient to be usefully applied in standardising any one of the various methods of treatment described as successful.

Treatment with electric light and the accompanying radiations.—Irradiation by electric arc or mercury-vapour lamps was tried by many of the earlier workers as a means of hastening maturity in uncertain seasons and for stimulating flower and fruit production in nurseries and green-houses. Hervé-Mangon (1861), Walkoff (1866), Siemens (*Nature*, 1880, **21**, 438, 456) and several later workers observed that photosynthetic activity proceeded in electric light just as efficiently as in sunlight. Dorsey (*Electrotech. Z.*, 1914, **35**, 236) experimenting with hothouse plants found that certain crops like lettuce were favoured by treatment with electric light while others like radish were not. Hayden and Steinmetz (*Gen. Elec. Rev.*, 1918, **21**, 232) observed that in artificial light, the rates of maturing and ripening of plants can be nearly doubled. In more recent years it has been recognised that both the arc and the mercury vapour lamps are not quite satisfactory because of their high but spasmodic intensity and their colour, which are somewhat injurious to plants. Harvey and Hendricks (*Bot. Gaz.*, 1924, **77**, 330) have investigated the limiting intensities and periods of exposure for a variety of crops. Mathews (Electro-Farming, 1928) found gas-filled filament lamps to be highly satisfactory.

It is difficult to state from the foregoing observations whether the beneficial effects noticed by previous workers were due to the light radiations, or to the ionisation of the air, or to any one of the attendant gaseous products like ozone, particularly when the source of illumination was an electric arc. Moreover, during the lighting, the major part of the electrical energy is dissipated as heat and the attendant rise in temperature would, no doubt, have influenced the vegetative growth as

well as the maturation of the greenhouse plants under experiment. Further knowledge regarding the mechanism of plant stimulation is therefore required before the process can be applied economically to obtain increased crop-production.

Utilisation of atmospheric electricity.—The treatment consists in conducting the energy from an elevated collector to either an electrode in the soil or discharge points above plants. Abbé Bertholon (1783) devised an apparatus, the electro-vegetometer, which consisted of a number of metal points supported above as in a lightning conductor leading below to an iron bar with a number of discharge points. By growing plants under the discharge points, he obtained highly satisfactory results with a large variety of crops. The plants thus treated made better vegetative growth and gave higher yields of the desired crops than those untreated (cited from Priestly, *Proc. Bristol Nat. Soc.*, 1907, **1**, 193). Grandeau (*Compt. rend.*, 1878, **78**, 60, 265, 939) adduced further evidence in favour of utilising atmospheric electricity by studying the development of big trees in forests and the consequent smothering of the smaller ones. He also demonstrated that plants protected from atmospheric electricity by overhead wire-netting did not develop properly and gave yields which were hardly 50 per cent. of those from plants grown in the usual way.

Abbé Bertholon's method found considerable favour in France where, in a modified form, it came to be known as the 'geomagnetifère' system. Treatment of plants with atmospheric electricity did not find much favour in either Britain or America.

With the scanty literature now available on the subject, it is difficult to state whether the method has possibilities of extensive application. The precise nature and mechanism of stimulation observed by Bertholon, Grandeau and others has yet to be understood. In view of the fact that thunderstorms in the tropics are frequent and often intense, it is possible that in India, concentration of atmospheric electricity over plants may lead to highly beneficial results. Further systematic study of the subject is, however, needed before the method can be safely advocated for use by farmers.

Earth currents.—Several workers in different countries have observed beneficial effects as the result of using earth-plates of dissimilar metals connected by conductors. The soil between the plates then becomes an electrolyte and a weak current flows through it giving the necessary stimulus to the growing plants. Speschnew (cited from *Gen. Elec. Rev.*, 1915, **18**, 14) obtained increased yields from various plants by the above treatment. Rauson and Le Baron (*Elec. World*, 1906, **47**, 1067) buried plates of copper and zinc at the opposite ends of lettuce beds and noted that the low voltage (0.5 V.) and current (0.4–15.0 milliamps) flowing between the

plates quickened the development of leaves so that the crops attained marketable size a week earlier than they otherwise would. An objection to this method suggested that although the previous workers obtained somewhat satisfactory results, it is possible that minute quantities of the metals passing into the soil either as rust or solution may ultimately prove toxic to the plants and poisonous to the consumers; thus leading to greater loss and trouble than the small advantage gained by earlier harvest.

Priestly (*Proc. Bristol Nat. Soc.*, 1907, **1**, 190) repeated the treatment under carefully controlled conditions and obtained not only healthy early crops, but also increased yields, amounting to 33 per cent. over the control in the case of beans.

Like some of the earlier methods, the one described above has not been tried extensively owing to the overwhelming popularity of overhead discharge methods; but it deserves further systematic study because of its cheapness, there being no expense additional to the outlay on metal plates. Moreover, in view of the findings of Hissinck (*Internat. Mitt. Bodenkunde*, 1922, **12**, 81) and others regarding base-exchange in soils, it is hardly possible that any more than traces of the toxic metals will pass into the growing plants. Earth-plates made of metals other than copper and zinc, preferably such cheap ones as occupy distant positions in the electromotive series, may be tried. It is also possible that by enclosing the plates in small earthenware pots, (*a*) loss due to rusting or action of salts in the soil may be minimised, and (*b*) chances of even minute quantities of the metals passing into the plants may be obviated.

The method of passing current from external sources through the soil has not, so far, met with much support. The treatment is stated to be more expensive than most others: scientific opinion regarding its utility is also somewhat conflicting. Cook (*Electrician*, 1898, **41**, 787) observed that the earth-current helped to increase the rate and proportion of seed-germination in the soil, but had no effect on either plant-development or crop-yield. Kövessi (*Compt. rend.*, 1912, **154**, 289) trying the same method, noticed that an earth-current was inimical to the germination of seeds. Gerlach and Erlwein (*Elektrochem. Z.*, 1910, **17**, 31) passed a 6-V. current (0.2 – 0.4 amp.) between iron electrodes buried at the opposite ends of barley and cabbage rows but derived no advantage from the treatment. Dorsey (*Electrician*, 1913, **72**, 442) tried the earth-current method at different voltages and current strengths, but was unable to obtain any significant increase in crop-yield. Peaslee (*J. Elec. Power and Gas*, 1914, **32**, 69) passed direct as well as alternating current of lower density than those used by previous workers between carbon electrodes and obtained

highly satisfactory, all-round increase in his crops. He obtained the best results at a power consumption of 0.5–0.6 W. per cu. ft.: there was earlier and increased percentage of seed-germination, more rapid and luxuriant vegetative growth with healthier and richer crop, especially of roots, as compared with the untreated plants. He reported that after treatment with direct current, the increase in the yield of cauliflower was about 150 per cent. and that of radish, over 400. Alternating current was also useful, but not so efficacious as the direct one. Many methods of earth-current treatment which are claimed to be improvements on the previous ones have been patented in recent years, but most of them have not been either described properly or tried extensively enough to warrant public confidence in their efficacy (Phœnix, *J.I.E.E.*, 1929, **67**, 1296).

It may be inferred from the instances cited above that although earth-currents have not always proved favourable, yet there are certain hitherto obscure conditions leading to increased crop returns. Definite knowledge with regard to the scientific basis of the treatment, with special reference to the response given by the different types of soils under various systems of cultivation and manuring is still lacking; and further work on the standardisation of procedure with individual crops is required.

Overhead discharge.—Passage of silent or glow discharge from overhead antennæ to the soil has been tried extensively in various countries and may, after being standardised, prove to be one of the cheapest methods of electro-culturing plants. The method has been studied in the laboratory as well as on the field. It offers considerable possibilities of success, though the results reported by various workers and, often, by the same workers in different seasons, have not been consistently satisfactory. The earlier experiments of Lemstrom (Electricity in Agriculture and Horticulture, 1904) were remarkably successful: increased yields ranging from 45 to 100 per cent. for various crops were noted. The results of the later trials by Lodge and Newman (*J.I.E.E.*, 1914, **52**, 333) were not so satisfactory, but held out promise of being useful under properly controlled conditions. Gerlach and Erlwein (*loc. cit.*) who followed the technique suggested by Lemstrom observed no difference between their treated and untreated plants: indeed, they found alternating current to be injurious to plant-growth. Stahl (*Elec. World*, 1911, **58**, 1549) on the other hand, found the overhead D.C. discharge to be an inexpensive method of quickening crop production. Gloede (*Elec. Rev. West. Elec.*, 1911, **59**, 975) obtained increased yields of flowers and fruit as the result of mild overhead discharge. He also noted that his electro-cultured fruits were sweeter than the untreated ones. Dorsey (*Electrotech. Z.*, 1914, **35**, 236) observed that the yields from the treated plants though high on the average, were not consistent. Peaslee

(*loc. cit.*) obtained generally enhanced yields from plants receiving nightly overhead discharge, but noted that the seasonal variations influenced considerably the quality and yields obtained. Britton (*Electrician*, 1918, **80**, 641) carried out a similar series of trials and observed that the plant response to overhead discharge was highly inconsistent and that the yields differed from those of the controls by + 23·5, + 153·0 and − 7·5 per cents. respectively. Hein (Phœnix, *loc. cit.*) experimented with brush discharges at 20,000 V. and obtained satisfactory increase in yields.

Since 1918, the Electro-culture Committee of England have investigated the various factors influencing plant response to electro-culture, particularly by the method of overhead discharge, and have noted the most suitable voltage, current density, height of the aerial, spacing between the wires, time and duration of discharge, treatment of the soil and certain of the physiological effects of the discharge (Interim Reports of the Electro-culture Committee, 1918–29; Blackman, *J. Agric. Sci.*, 1924, **14**, 240; Blackman and Legge, *J. Agric. Sci.*, 1924, **14**, 264). Contrary to the earlier findings, alternating current was observed to be just as efficient as direct, and even superior to it in certain cases.

The field experiments were pursued through many seasons and at various centres, the technique being improved at every stage to minimise adventitious errors, but the results proved inconsistent. In the same season, with apparently the same treatment, satisfactory plant response was observed at some centres and almost none at others: the results at the same centres in successive seasons were also inconsistent. The meteorological conditions doubtless influenced the crop returns, but they were not entirely responsible for the discordant results from electro-cultured plants. The soil conditions also appeared to affect the results unaccountably. Recently, Blackman and his co-workers carried out some carefully planned experiments at Rothamsted and elsewhere, but the results were highly erratic, casting doubt on the practical significance of the observations collected so systematically and patiently during many years past.

The foregoing observations, though somewhat discouraging, only emphasise the need for further work. There are obviously certain hitherto unsuspected factors affecting the success of overhead treatment, and these must be ascertained before further progress can be achieved.

Electrical treatment of Seeds, Seedlings and Plants.

Fry's patented method of activating seed (*U. S. Pat.*, 1,106,039) consists in packing them between metallic electrodes in a 10 per cent. solution of a nitrate, or ammonium sulphate, or any one of the soluble

salts which are commonly used as fertilisers, then passing current from the main for a number of hours, after which the seeds are taken out, dried and supplied to farmers. Dunn (*Elect. Rev.*, 1919, **84**, 89) reported the process to be highly useful in hastening germination and stimulating tillering. The advantages claimed could not be confirmed by other workers in England; Sutton, who carried out extensive series of trials, concludes that the method is of doubtful value and does not justify the expenses involved (cited from Phœnix, *loc. cit.*).

Favourable reports regarding the possibilities of seed-treatment have been received from Australia, however. Bennett's method (*B.P.* 268,291) consists in rolling seeds, previously soaked in water to soften the cuticle, with a cylindrical vacuum tube containing a Tesla coil. By its adoption the benefits of increased germination percentage, greater yield and improvement in quality have been claimed for a variety of crops. The method has not been sufficiently tried outside Australia, and further trials under a variety of soil and climatic conditions are required before it can be adopted extensively.

Nehru (*Proc. Indian Sci. Congress*, 1930, 38) energised a variety of seeds by passing high tension electric sparks (1,000—10,000 V.) over them for 5 to 10 minutes. As the result of the treatment he obtained quicker germination, better growth, increased yields and resistance to certain plant diseases and insect pests.

Energising plants by passing an electric current through them does not appear to have met with much success: Marx, who passed current through *Elodea canadensis* soaked in water observed no marked benefit, but noted frequent depression in development (*Ann. Bot.*, 1929, **43**, 163).

The foregoing examples show that further study of seed and plant-energising is required before any of the processes can be applied extensively. The effect of the current on the various chemical constituents and the enzymes of the seed has to be studied. The influence of the treatment on assimilation and respiration, and various vegetative and reproductive activities of the growing plants have also to be clearly understood before a rational application of any one of the different methods of energising can be adopted successfully.

High Frequency Currents.—Bennett's method (*loc. cit.*) of seed-energising has been already described. Benedetti (*Atti R. Accad. Lincei*, 1926, **4**, 324) studied the effect of electromagnetic fields oscillating at high frequencies on some germinating seeds and noted that under suitable conditions the treatment hastened seed-germination and favoured the development of seedlings. Mezzadroli and Vareton studied the effect of short-wave astral rays on the germination of seeds and the growth of plants, but did not obtain encouraging results (cited from *Int. Rev.*

Agric., 1929). Homberger (*Bull. Agric. Intelligence*, 1930, **11**, 1430) observed that unlike high tension currents, those of high frequency have no harmful effects on plants. Experiments conducted in America at 10,000 V. and a frequency of 200,000 per sec. gave increases in yield amounting, in certain cases, to 75 per cent. The treated plants were also noted to be richer in chlorophyll than the untreated, and the method is obviously quite promising.

Nehru (*Proc. Indian Sci. Congress*, 1930, 38) has recently observed that the soil can also be energised by a 'tickler' or a discharge tube through which air is pumped and sparked at the orifice; the conical nozzle is driven into the soil at different positions and the sparked air pumped for 2–5 minutes at a time. He combines this treatment with that of seed-energising which has already been described, and obtains satisfactory results. The method appears to be promising and deserves more extended investigation in both the laboratory and the field. The details are at present somewhat elaborate and difficult for the average farmer to follow, but it is possible that the technique could be simplified and the details standardised to render the method more easily workable.

The nature of the action of electricity on plants.—The mechanism of the action of electricity on plant life is still not properly understood. Numerous theories have been propounded by various authors from time to time, but most are discordant. From his studies on forest trees, Grandeau (*loc. cit.*) concluded that atmospheric electricity is just as essential to plant-development as sunlight, air and water; he could not explain its role but was convinced of its necessity for plant life. Pollacei (*Atti Inst. Bot.*, 1905, **2**, 7) observed increased assimilation of carbon dioxide and plant nutrients resulting from an electric discharge over plants. Bach (*Compt. rend.*, **26**, 479) obtained evidence that formaldehyde is generated in the atmosphere through which an electric discharge passes and argued that for this reason electrical treatment is beneficial. Euler (*Ber.*, 1904, **37**, 3415) repeated Bach's work, but could not detect formaldehyde. Various other authors detected ozone with various active ions, and attributed to them the action of electricity on plant-growth. Lemström (*loc. cit.*), Priestly (*loc. cit.*), Escard (*Rev. Gen. Sci. pur et app.*, April 30, 1913), Peaslee (*loc. cit.*) and many other workers have studied the botanical significance and the physiological effects of electric discharge on plants, but their investigations have not thrown much light on the precise mechanism of the action. Waller (*Proc. Roy. Soc.*, 1901, **B**, **162**, 129) studied the electrical results of light on plants and observed that the leaves exhibit electromotive effects and after-effects amounting to fractions of a volt depending on the extent of illumination. He also noted that the chloroplasts are concerned in the development of electromotive force in the plants. Using extremely sensitive instruments of his own design, Bose (e.g., Plant response as a means of physiological investigation,

1906) has demonstrated that every phase of activity in plant life is associated with changes in the electrical properties of the various tissues, and may at times even result in a flow of current.

The foregoing observations no doubt suggest that external electrical stimulus is likely to play a part in influencing plant development and activity. They do not however indicate the biochemical mechanism by which the simpler physiological processes like photosynthesis, respiration, assimilation and translocation of nutrients are effected. Stone (cited from *Bull. Agric. Intell.*, 1921, **12**, 262) observed that following a discharge through the space above an area of soil, the bacterial numbers become sixfold. Mathews (*loc. cit.*), who carried out extended field trials for many years, inclines to the view that electrical energy helps to stimulate the assimilation of nitrogen by plants, an observation which indirectly accords with that of Stone, because increased numbers of bacteria will lead to greater release of plant food, particularly nitrogen, in available form from the soil. The Electro-culture Committee (Interim Reports, 1920–29, Nos. I—X), after an extended series of trials at many centres and spread over several years have come to the conclusion that electro-culture has no effect on the plant response to nitrogen: indeed, they found that nitrogen in excessive amounts is definitely unfavourable to the discharge. They also noted that a high phosphate content actually depresses the yield from electro-cultured plants, but that the injurious effect is reduced in presence of higher amounts of potash and nitrogen. Reporting on his experiments in 1922 (fifth report of the Electro-culture Committee, 1923, 17) Blackman concluded that electrical energy has practically no effect on the vegetative activity of the plant, but influences the reproductive activity to a considerable extent. Thus, although the yield of grain from electro-cultured barley was generally higher than that from the untreated seed, the total dry weight of plant was practically the same in both the cases. He also observed that there was no increase in the total number of flowers, but that the electro-cultured plants had fewer sterile flowers than the control. Moreover, the treated plants carried fewer seconds or shrivelled grains, and the weights of grain were individually and totally greater than those from the control plants. The foregoing observations are somewhat discordant with those of the previous workers, many of whom have stated that one of the most marked effects of electro-culture is the increased tillering and vegetative growth.

Haynes (*Sci. Progress*, 1923, **18**, 223) suggests that change of reaction in the tissue-fluids and increased permeability of membranes are the causes of electrical stimulation. His observations are suggestive, but do not help to explain the peculiar variations that have been noted with regard to both plant-development and crop-yields from season to season on the same experimental areas. Thus they cannot explain the

remarkably striking observations of Britton (*loc. cit.*) who obtained yields varying from + 153·0 to − 7·5 per cent. as compared with the untreated plots. Several authors have considered the effect of electricity to be indirect, but have not adduced sufficient evidence to support their theories. On the other hand, Blackman, Legge and Gregory (*Proc. Roy. Soc.*, 1924, **95**, 214) observed that the development of the coleoptile of barley was greatly stimulated by passage of very low density current (10,000 V., $0·5 \times 10^{-10}$ amp.) and noted that the effect persisted for 4 hours after treatment. Changing the current direction produced the opposite effect, and they infer that the effect was due to the direct action of the current itself, and not to any of the products of the discharge. Studying the effect of electric discharge on the respiration of barley, Middleton (*Ann. Bot.*, 1927, **41**, 345) observed this to be increased by about 30 per cent. in ionised air. The degree of stimulation varied with the extent of ionisation. The trials also showed that the effect was due to the active ions themselves and not to any of the products of ionisation. Whimster (*Ann. Bot.*, 1927, **41**, 357) came to a similar conclusion from his study of the rates of assimilation and respiration of *Pelargonium zonale*: he also observed that the stimulus continued for appreciable lengths of time even after removing the ionised air from around the plants.

It has already been pointed out that the season plays an important part in determining the success or failure of electro-culture. This influence is determined by the time and extent of rainfall, humidity, hours of bright sunshine, maximum and minimum temperatures. Any or all of such factors may determine the plant response to electro-culture; it is therefore desirable to study the effects of the different climatic factors individually and to ascertain which of them is individually responsible and to what extent. In this connection, the observations of Höstermann (*Electrotech. Z.*, 1910, **31**, 294) regarding the beneficial effect of foggy weather deserve further systematic study from the chemical as well as biological point of view.

It has been noted by several workers that in certain seasons the electro-cultured plants failed, while the control developed normally. Such results could not have been due merely to unfavourable weather detracting from the beneficial effects, for then the control plots should have yielded less than the electro-cultured ones. The observations suggest that changes in one or more of the seasonal factors actually determine whether electro-culture will be beneficial or not in a particular season. It is also possible that when the treatment is given on a number of days in succession, the effect is favourable on certain days and unfavourable on others.

Our knowledge of the biochemical aspect of electro-culture with reference to both soil and plant is still obscure. Extension of Stone's

observation regarding increase in bacterial numbers (*loc. cit.*) will show whether the transformation of plant nutrients proceeds alike in all soils when subjected to the influence of electric discharge. Study of changes in the nature and numbers of different other forms of microflora should also prove useful. Important biochemical processes such as oxygen absorption and carbon dioxide evolution, ammonification and nitrification should be followed to ascertain whether changes in the composition and character of microflora are accompanied by the hastening of such processes. The study of the biochemical response of the plant will help to explain (*a*) the significance of the treatment, (*b*) the causes of the abnormally high or low yields observed in some cases and (*c*) the nature of the resistance offered by the electro-cultured plants to certain fungus diseases as well as insect pests.

The observations of the Electro-culture Committee appear to suggest that the treated plants do not respond to manurial treatment, particularly to artificials. It is still not definite whether such observations would apply to different types of organic manures as well as to the different soils commonly met with under the tropical conditions. When these are favourable, depletion of minerals and nitrogen from the soil would no doubt follow. Since the extent of availability of such nutrients is a limiting factor in crop-production, it may be inferred that although the electro-cultured plant did not respond to nitrogen and phosphate in the trials carried out by Blackman and his co-workers, it would do so under more depleted conditions of the soil such as may be expected after a succession of crops without adequate manuring.

Many of the earlier workers have observed that electro-culture is not beneficial when applied in bright sunlight. Lemström found night treatment to be useful. Henrici (cited from Whimster, *loc. cit.*) observed that ionisation of air aided assimilation in dull weather, but retarded it in bright sunshine. Blackman and his co-workers found the morning hours to be suitable for the treatment, but noted that the night discharge was of doubtful value and might even prove harmful to plant-growth. All the previous workers are agreed that treatment during periods of severe drought, or bright sunshine, or heavy rain should be avoided as they are likely to affect plant-development. The time of treatment is of only relative significance however. Thus, 6 a.m. which is mentioned by Blackman to be a suitable hour for England may be brightly sunny and even hot in a tropical country. It is therefore desirable to define the time in terms of sunshine intensity, cloudiness or otherwise, temperature and relative humidity rather than by the clock.

The conditions leading to increased assimilation by electro-cultured plants have not, so far, been studied systematically.

Whimster (*loc. cit.*) observed that when the leaves of *Pelargonium zonale* are placed in ionised air, assimilation is not increased appreciably, but that the respiration is hastened by over 80 per cent. It is however doubtful whether the above observation is applicable to the whole plant. Field trials by several workers show that under favourable conditions increased assimilation resulting in all-round plant-development may be expected; and further study of the various factors such as degree of ionisation of air, relative humidity, intensity of light and concentration of carbon dioxide on assimilation and translocation is required.

Several authors have stated that electro-cultured crops are superior in quality to those untreated, but various analyses have not so far shown anything abnormal with regard to the chemical composition of the former. Recent investigations on the digestibility and nutritive values of different food materials have shown, however, that the chemical composition is often apt to be misleading. Animal experiments with regard to the relative growth-promoting qualities of the treated as well as untreated crops should therefore be conducted to settle this question. It is probable that at least some of the electro-cultured products may possess better taste and flavour than those untreated, and appeal by such qualities to consumers: others may be rich in vitamins and contain minute quantities of the various growth-promoting substances which add to the food-value of such materials. If the electro-cultured materials possess such qualities, they will be more desirable than the untreated ones in spite of the uncertainty of success attending the methods practised at present. The high prices commanded by superior quality should more than compensate for any possible loss due to depression in yield. This aspect of the subject also deserves systematic study.

In the present series of investigations, it is proposed to (*a*) study some of the more fundamental problems suggested in the foregoing pages and thereby ascertain the nature and extent of the various influences affecting plant-development during electro-culture, and (*b*) standardise a system of treatment that will increase the yield of crops of the desired quality under various conditions of soil and climate, with special reference to those of India.

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STUDIES IN ELECTRO-CULTURE.

Part II. Influence of Electrical Treatment on the Germination of Barley and the Diastatic Activity of Malt.

By K. Venkata Giri and V. Subrahmanyan.

Butterfield, Barclay and other earlier Australian workers conducted systematic work on the stimulation of seeds by electricity (Mackinnon, *Sci. and Ind.*, 1920, **2**, 24). They observed that barley soaked in a solution of copper sulphate could be electrified with an ordinary medical coil in 3–5 minutes, and that plants raised from the seeds thus treated, gave about 40 per cent. more yield than the controls. Fry developed a system of electrical treatment which was subsequently patented as the 'Wolfryn' process (*U. S. Pat.*, 1,106,039) and which excited considerable attention in both England and Australia. Reporting on the results of the treatment, Dunn (*Elec. Rev.*, 1919, **84**, 89) mentions that quickened germination, prolific tillering, increased yields of richer grain, sturdier straw and resistance to a variety of diseases may be expected by the use of the Wolfryn-treated seeds. Others were not so hopeful. Thus, Brenchley could observe no difference between plants raised from treated seeds and those from untreated. Gerrard reported that trials carried out with electrified seeds at Wye resulted in failure. The investigations of the British Board of Trade suggested that the beneficial effects of the treatment might have been largely due to the preliminary soaking of the seeds in the electrolyte (*Elec. Rev.*, 1919, **85**, 377). Various other systems of seed treatment have lately been developed, among which mention may be made of the method of Bennett (*Brit. Pat.*, 268, 291) as the result of which highly satisfactory yields of grain and straw are claimed to have been obtained.

The precise nature of the action of electricity on seeds is still not understood, though various theories based on the ionisation of the electrolyte, permeability of the seed, osmotic pressure of the solution, and sterilisation of the seed-coat have been advanced by different workers. Very little is known also regarding the physiological response of the seed to electrical treatment. It is possible that the passage of current through the seed brings about some physical or chemical changes which influence certain phases of living activity such as germination, development of the seedling, response to plant nutrients, assimilation and translocation. Systematic study of such physiological effects is required before the significance of the various field observations cited above can be properly understood.

In the present investigation, some aspects of the changes occurring during the germination of electrically treated barley have been studied. Barley was chosen because (*a*) consistently favourable reports regarding the beneficial effects of so treating it have been received, no less than 30–40 per cent. increase in yield being claimed by many observers, and (*b*) the changes attending the germination of barley are of considerable economic importance and have already been studied, in great detail, by different workers from other points of view. It was therefore thought that the significance of the various changes attending the germination of electrically treated seed can be better understood in the case of barley than in that of any other grain.

The most conspicuous changes attending the germination of barley are the activities of the different enzymes resulting in the transformation of starch into dextrins and sugars and of protein into proteoses and amino-acids; and the movement of the various products into the seedling, which is thus provided with its early nutrition. The effects of the treatment were therefore sought from (*a*) the rate of germination, (*b*) the diastatic and the proteolytic activities of the germinating seed at various stages and under different conditions of electro-culture, and (*c*) the development of the seedling.

In view of the almost fanciful prices paid for 'quality' in malting barley, considerable attention has recently been paid by various workers to that subject. To the maltster, the value of barley depends largely on the amount of extract in the wort prepared from it. The investigations carried out under the auspices of the Institute of Brewing Research Scheme (Rothamsted Conference on Malting Barley, 1928; Bishop, *J. Inst. Brewing*, 1930, **36**, 352) have shown that the yield of extract is, generally, inversely related to the nitrogen content of the malting barley. Field experiments carried out during the past ten years at Rothamsted and elsewhere (Russell, Agricultural Research in 1929, 1930, 144–145), have shown that where the soil cannot supply all the nitrogen required by the plant, the yield of barley, without impairing the quality, can be increased by judicious application of nitrogenous fertilisers. It is not, however, generally possible to control the nitrogen uptake of the grain because this is determined, not by the absolute quantities added to the soil, but by the amounts rendered available under the existing soil and climatic conditions. It is also influenced by the available amounts of the other plant nutrients, such as phosphate and potash present in the soil. Moreover, there are several tracts of land, like the Fen districts of England, where the soil is so rich in nitrogen that even without the addition of nitrogenous manure, the amount of nitrogen passing into the grain is so high that its quality is seriously impaired. The malt prepared in the usual way from such barley contains, invariably, a high percentage of nitrogen and yields a wort of poor quality.

The observations of earlier workers regarding the quicker and the more efficient germination of electrically treated seed, together with those of Punnet (*Biochem. Bull.*, 1913, **2**, 555) and Lob and Sato (*Biochem. Z.*, 1915, **69**, 1) regarding the favourable influence of ionised air on enzyme action suggested that, during the germination of the treated barley, there may be quicker transport of nitrogen into the seedling than in the case of the untreated. It therefore seemed probable that if the activity of the protease in the electrically treated seed is greater than that in the untreated, the malt prepared from the former will contain less nitrogen and give a larger yield of wort of the desired gravity than the latter. In other words, it appeared probable that the quality of malting barley may be improved by electro-culture.

With a view to testing the above hypothesis, the nitrogen content of malts prepared from treated barley and the gravity of their worts were determined at various stages.

EXPERIMENTAL.

Method of treatment.—Malting barley of the Plumage Archer variety, obtained from the United Breweries of Kaiti in the Nilgiris, was used for the present series of trials. Before treatment, the seeds were soaked first in water for 24 hours and then in sodium nitrate solution of known concentration for a definite length of time. They were then packed between chemically pure carbon electrodes in glass cells and electric current (A.C., 25 cycles, 210 V.) of varying densities passed through them for different periods. A stream of salt solution was kept gently circulating through the cells to preclude the otherwise inevitable rise of temperature, and to maintain the current strength constant throughout each experiment. At the end of the specified period, the seeds were removed, washed carefully from the adhering salt and germinated at a constant range of temperature (24–26°). Representative specimens of the germinating grains were removed at intervals of 24 hours and dried by stages, at temperatures rising from 50° to 95°. The diastatic activities of the different preparations of malt thus obtained were compared with those prepared under the same conditions from similar specimens of barley after merely soaking them in water and salt solution respectively. The diastatic powers were determined by Ling's volumetric method as modified by Hind, Threadgold and Arnold (*J. Inst. Brewing*, 1926, **32**, 26).

General effects of the electrical treatment.—Barley was first steeped in water for 24 hours and then in 1 per cent. solution of nitrate for

2 hours, followed by passage of current ($7{\cdot}1 \times 10^{-3}$ amp. and $8{\cdot}6 \times 0^{-3}$ amp.) for 2 hours. The diastatic powers of the malts obtained from the treated specimens were compared with those prepared after being steeped in water and salt solution respectively.

In the course of preliminary trials it was observed that the passage of comparatively heavy current caused the temperature of the electrolyte to rise rapidly. The barley thus treated germinated in 12-24 hours, chiefly as the result of the high temperature of the electrolyte; intermittent passage of current was next tried, but did not prevent the rise in temperature. Passing a continuous stream of electrolyte through the cells was found useful in maintaining a uniform temperature and thus helping to bring about a normal germination. The malts prepared from the different specimens dried at about the same rates, but it was noted that those electrically treated developed a sweeter flavour.

A study of the diastatic power of the different preparations (Fig. I) shows that the treated specimens developed activity more readily than the others. The latter, however, made up for the difference within 48 hours, so that after that period there was no significant difference between the two sets of preparations. Later trials showed that the current densities used in the above experiment were too high. It was also found advantageous to reduce the concentration of the electrolyte.

Effect of current density on diastatic activity.—The seeds were soaked in a dilute solution ($0{\cdot}1$ per cent.) of the electrolyte and treated with current of different densities, for two hours in each case, the electrolyte being kept circulating as before. The results (Fig. II) show that a comparatively weak current ($1{\cdot}7 \times 10^{-3}$ amp.) is almost without effect on the diastatic activity. Slightly stronger currents ($2{\cdot}86 \times 10^{-3}$ amp. and 430×10^{-3} amp. respectively) evoke very much betterresponse and lead to considerably enhanced diastatic activity, but further increase in current density to $7{\cdot}1 \times 10^{-3}$ amp. is without any apparent effect.

It is difficult to explain, at this stage, the significance of the disproportionate effects of the different current strengths. It is possible that a very weak current does not supply sufficient energy to activate the enzyme. The distinct rise in activity noticed at the end of 24 hours in the case of seeds treated with $7{\cdot}1 \times 10^{-3}$ amp. shows that the enzyme is, to some extent, stimulated by the treatment: there is, however, no marked rise observed after that period while in the case of seeds treated with $2{\cdot}86 \times 10^{-3}$ amp. and $4{\cdot}3 \times 10^{-3}$ amp. respectively, the favourable effect is most prominently seen between 48 and 96

hours. The results suggest that after producing the initial effect, the denser current leads to a reaction which hinders further rise in activity.

It may be mentioned, in this connection, that the different observations recorded above are in keeping with those of many other biological phenomena which respond suitably only under such physical conditions as are their specific optima.

Period of treatment as affecting diastatic activity.—Seeds soaked in a 0·1 per cent. solution of sodium nitrate were treated with currents of $1{\cdot}7 \times 10^{-3}$ amp. and $4{\cdot}3 \times 10^{-3}$ amp., which were interrupted after 1, 2, and 3 hours, respectively. They were then washed, spread out to germinate and the diastatic activities determined at different subsequent intervals.

The results (Fig. III) show that up to the end of 48 hours there was no significant difference between the effects of the various treatments. After that period, the specimens treated with $4{\cdot}3 \times 10^{-3}$ amp. showed very much greater activity than the others and continued to do so almost to the end. Treatment with $4{\cdot}3 \times 10^{-3}$ amp. for 1 hour was less effective than that for 2 hours: in the former case, the activity showed a distinct decrease at the end of 96 hours, while in the latter, it continued to rise up to 120 hours. Passage of the current for 3 hours was not, however, so beneficial as that for 2 hours: in the former case, the activity was somewhat depressed in the earlier stages, and although it showed a distinct rise in the later ones, it was at no time so high as in the latter. It may, therefore, be inferred that the maximum increase in diastatic activity can be obtained by treatment for 2 hours at $4{\cdot}3 \times 10^{-3}$ amp.

Observations on seeds treated for 1 and 3 hours respectively with $1{\cdot}7 \times 10^{-3}$ amp. show that there was no significant response in either case, the diastatic activities of their malts being at no time appreciably different from those of the untreated ones. Even prolonged treatment with a current strength below the optimum does not, therefore, appear to be beneficial.

The mechanism of stimulation of diastatic power.—The increased activity resulting from the passage of an electric current under optimum conditions may have been due to one or more of the following causes:—(*a*) Fundamental change in the composition of the substrate, (*b*) stimulation of the enzyme present in the germinating seed, and (*c*) larger amounts of the enzyme being developed by the treated seed.

A study of the progress of hydrolysis shows that the electrical treatment could not in any way have affected the composition of

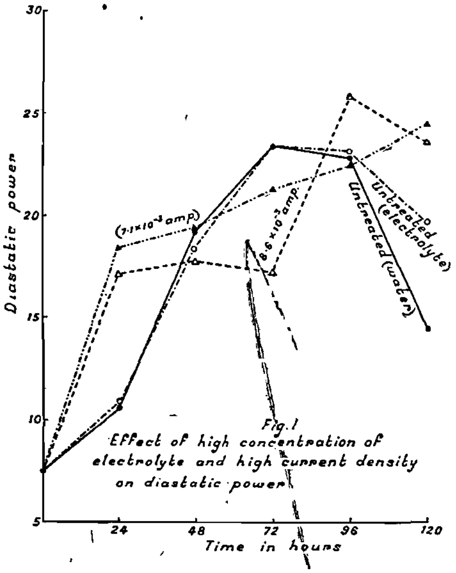

Fig. I
Effect of high concentration of electrolyte and high current density on diastatic power

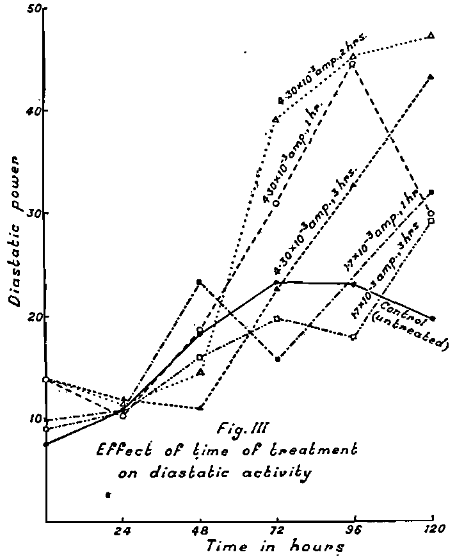

Fig. III
Effect of time of treatment on diastatic activity

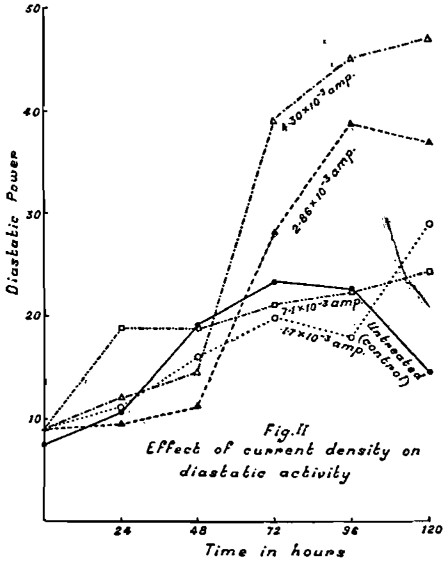

Fig. II
Effect of current density on diastatic activity

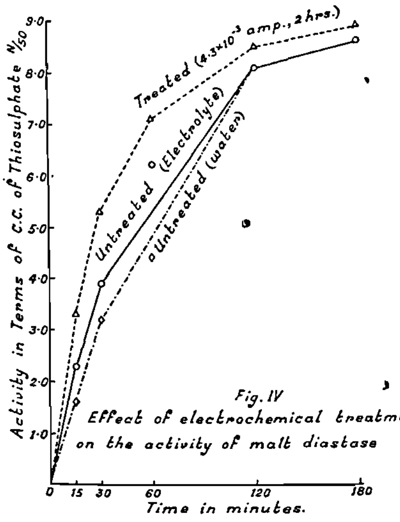

Fig. IV
Effect of electrochemical treatment on the activity of malt diastase

barley starch, since in that case the diastatic activity of the treated malt should have been higher than that of the untreated, throughout the experiment. The fact that the diastatic powers were almost the same in all the cases for the first 48 hours suggests, on the other hand, that only the enzyme was affected.

To determine whether the electrical treatment led to increased activity of the enzyme unit, the following experiment was made:—Specimens (60 g.) of treated as well as untreated malts were shaken with distilled water (180 c.c.) for 6–8 hours and the mixtures filtered first through cloth and then through paper pulp. The filtrates were centrifuged and the solid contents of the clear extracts determined. After dilution with water sufficient to render the solid contents the same in all the cases, the diastatic activities of the different extracts were determined. To 50 c.c. lots of 1 per cent. starch solution, 10 c.c. of Walpole's acetate buffer of P_H 4·6 and 2 c.c. of enzyme extract were added and the mixtures maintained at 35° in a thermostat. Aliquot parts of the mixtures were removed at stated intervals and their sugar contents estimated by the method of Shaffer and Hartmann (*J. Biol. Chem.*, 1920–21, **45**, 365).

The results (Fig. IV) show that although the dialysed enzyme from treated malt is slightly more active than that from the untreated, the difference is not so considerable as to account for the observations of the earlier experiments. Since it has already been shown that the extract from a unit weight of the treated malt brings about a larger transformation of starch in a given time than that from the untreated, it should be inferred that the former contains a higher concentration of either the enzyme or the necessary activators, or both. In view of the fact that only the crystalloids in the extracts were removed by dialysis, the activators probably present in larger amounts in the electrically treated malt might have belonged to that class.

Some doubt has, however, been recently cast on the existence of any definite group of substances capable of activating vegetable amylases. Sherman and Thomas (*J. Amer. Chem. Soc.*, 1915, **37**, 623) and Sherman and Walker (*J. Amer. Chem. Soc.*, 1919, **41**, 1867; 1921, **43**, 2461) found that addition of neutral salts and amino-acids led to increased rates of starch hydrolysis by amylases of vegetable as well as animal origin. On the other hand, Koga (*Biochem. Z.*, 1923, **142**, 159) observed that neutral salts were without any effect on vegetable amylases, while Fricke and Kaja (*Ber.*, 1924, **57**, 310, 313) noted that they actually inactivated electro-dialysed diastase. Patwardhan and Karmarkar (*J. Indian Inst. Sci.*, 1930, **13A**, 159) recorded that amino-acids were without any effect on wheat amylase and, in a later unpublished investigation showed that these had no influence on the activity of

most vegetable amylases examined by them. In view of the above and the fact that the different preparations of malt examined in the present investigation were obtained from the same specimen of barley, it appeared to be hardly probable that any particular activator developed in the electrically treated seed was responsible for the increased diastatic power.

The results of the investigations of the British Board of Trade (*loc. cit.*) suggest that at least a part of the beneficial effect attributed to electrical treatment may be traced to preliminary soaking in the electrolyte. Although the control experiments of the present series showed that mere soaking in the electrolyte led to no response, it was still considered possible that, on passing the current, active ions might have penetrated the seed-coat and brought about, either directly or indirectly, the increased diastatic activity.

To determine whether traces of the nitrate were present in the malt of treated barley, the following experiment was made:—Specimens of dry malt were powdered and extracted with large excess of distilled water. The extract was passed through a coarse filter and the filtrate treated with cupric hydroxide (Van Slyke, *J. Biol. Chem.*, 1917, **32**, 455) to remove sugars and other organic substances. The clear and colourless extract was concentrated to small bulk and then tested for the presence of nitrates with phenoldisulphonic acid and diphenylamine, respectively. The tests were negative, thereby indicating that any nitrate originally adhering to the barley was either removed by the thorough washing following electrical treatment, or transported to the plumule and the radicle during germination.

The aqueous extract of treated malt was found, after dialysis, to contain 71·8 mg. of solid per 100 c.c. as compared with 45·8 and 52·2 mg. present in the water and the salt-treated specimens respectively. This observation and the previous experiments suggest that the apparently higher diastatic power of the extract from electrically treated malt is due to a greater concentration of the enzyme therein.

Liquefaction and saccharification of starch.—During recent years, considerable evidence has shown that vegetable amylases contain at least two components, the liquefying and the saccharifying enzymes, which (*a*) can be at least partially separated from each other and (*b*) respond differently to a variety of physical and chemical treatments (Wijsman, *Rec. trav. chim.*, 1890, **9**, 1; Pottevin, *Ann. Inst. Pasteur*, 1899, **13**, 665; Frankel and Homburg, *Biochem. Z.*, 1906, **8**, 389; Fricke and Kaja, *loc. cit.*, Narayanamurti and Norris, *J. Indian Inst. Sci.*, 1928, **12A**, 134). To ascertain whether electrical treatment had favoured the increased development of either or both the liquefying

and the saccharifying components, the following experiment was made :—To specimens (5 g.) of treated and untreated malt, 150 c.c. of water was added, and after being vigorously shaken, the mixtures were treated with toluene, allowed to stand for 12 hours, filtered and the filtrates centrifuged from suspended matter. Aliquot parts (10 c.c.) of the enzyme solutions were added to mixtures of 40 c.c. of potato starch (2 per cent.), 10 c.c. of Walpole's acetate buffer (P_H 4·6) and 20 c.c. of water. The reaction mixtures were then maintained at 34° and their changes in viscosity as well as sugar content determined in the manner described by Patwardhan and Norris (*J. Indian Inst. Sci.*, 1928, **11A**, 123).

The results (Fig. V) show that the amylase from treated barley effected liquefaction and saccharification of starch more rapidly than that from untreated grain. The effect of the electrical treatment was slightly more pronounced in the case of the liquefying than the saccharifying component, particularly in the later stages. The differences were not however so great as to suggest that the liquefying enzyme was either stimulated to a greater extent or present in larger proportion than the saccharifying component. In view of the previous observation relating to the concentration of the enzyme, the results of the present experiment suggest that the increased diastatic activity observed in the case of electrically treated malt was due to larger amounts of both components.

Effect of electrical treatment on nitrogen transformations during malting.—The observations of Bishop (Ph. D. thesis, Cambridge; *J. Inst. Brewing*, 1929, **35**, 323) have shown that during both the development of the barley grain on the plant and the subsequent process of malting, there is a definite relationship between the total nitrogen of the grain and its distribution into various forms. In view of the above and the earlier observations regarding the relationship of nitrogen to quality in malt, it was considered desirable to determine the effect of electrical treatment of barley on the nitrogen contents of malts prepared at different stages.

The germination of barley as well as the drying of the specimens were carried out in the manner described already. The nitrogen contents of the different preparations of malt were determined by the kjeldahl method (A.O.A.C., 1925). The results (Fig. VI) show that the electrical treatment had a marked effect on the nitrogen content of malt. Whereas the water and the salt-soaked specimens lost nitrogen to the extent of only 0·23 and 0·26 per cent., respectively, in the course of 96 hours, the treated malt lost 0·58 per cent. within the same period. Since none of the nitrogen could have passed outside the plant system and since the plumules and the radicles derived their

nutrition exclusively from the seed, it may be inferred that the nitrogen lost by the malt was actually gained by the seedlings (Table I).

TABLE I.

Percentages of barley nitrogen passing into the seedlings at different stages during germination.

Time in hours	Percentage of total nitrogen		
	Treated	Untreated	
		Electrolyte	Water
20	11·0	6·1	3·9
48	14·3	6·1	5·5
72	...	6·1	...
96	32·0	17·0	12·8

The movement of nitrogen (Fig. VI) proceeded comparatively slowly during the first three days, the rate being almost insignificant between 24 and 48 hours. After that period, it became suddenly rapid and the quantity passing out into the plumule and the radicle within the next 24 hours was more than the aggregate passing out during the three previous days. Under the experimental conditions, the movement proceeded about twice as fast in case of the treated seed. In view of the fact that the uptake of the essential nutrients, particularly nitrogen, by the seedling determines the various physiological activities such as assimilation, growth and tillering, it may be inferred from the above that the electrical treatment should prove helpful at least in the early stages of plant life.

It is not yet possible to explain the mechanism of increased uptake of nitrogen by the seedling. It may be that (*a*) there was greater proteolytic activity in the treated barley than in the untreated, resulting in quicker breakdown of proteins and transport of the products into the seedling, or (*b*) the electric current imported a physical stimulus or introduced a physico-chemical condition resulting in the seedling germinating from treated barley exerting greater osmotic pressure. If the latter be the case, then the effect may be expected to persist longer and lead to the seedling raised from electrically treated barley responding better to manurial treatment on the seed-bed than the one from untreated barley. Further work is needed to settle this point.

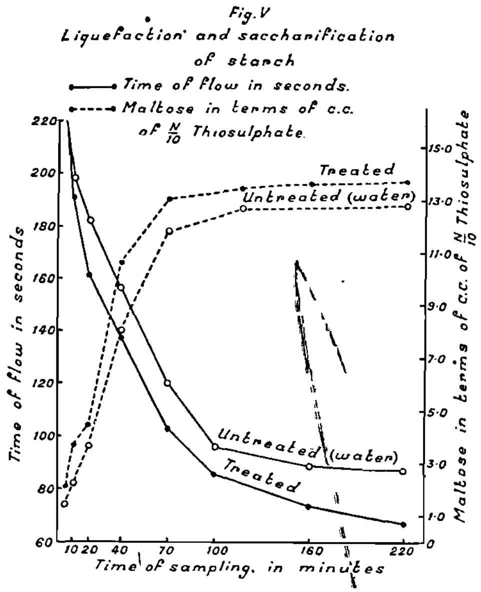
Fig. V
Liquefaction and saccharification of starch
Time of flow in seconds.
Maltose in terms of c.c. of N/10 Thiosulphate.
Treated
Untreated (water)
Untreated (water)
Treated
Time of flow in seconds
Maltose in terms of c.c. of N/10 Thiosulphate
Time of sampling, in minutes

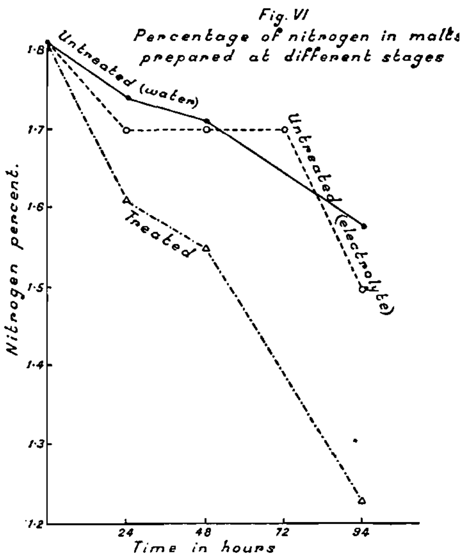
Fig. VI
Percentage of nitrogen in malts prepared at different stages
Untreated (water)
Untreated (electrolyte)
Treated
Nitrogen percent.
Time in hours

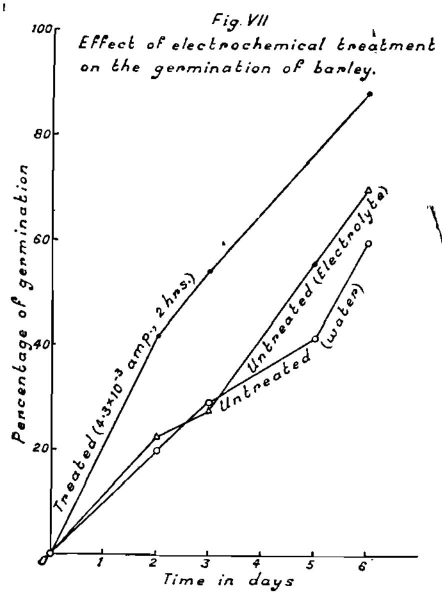
Fig. VII
Effect of electrochemical treatment on the germination of barley.
Treated (4.3×10^-3 amp., 2 hrs.)
Untreated (Electrolyte)
Untreated (water)
Percentage of germination
Time in days

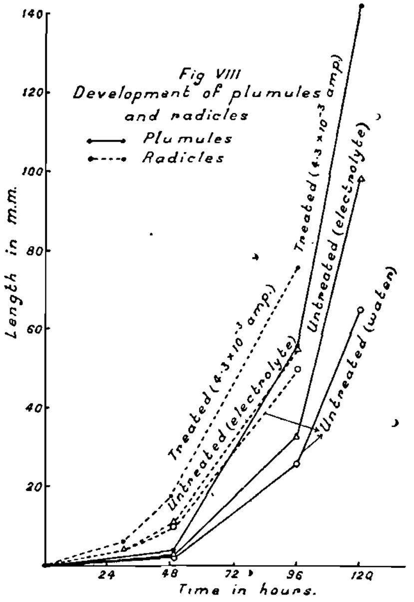
Fig VIII
Development of plumules and radicles
Plumules
Radicles
Treated (4.3×10^-3 amp)
Untreated (electrolyte)
Untreated (water)
Treated (4.3×10^-3 amp.)
Untreated (electrolyte)
Length in m.m.
Time in hours.

The researches carried out under the auspices of the Institute of Brewing have already shown that when adopting the usual system of steeping and malting, the nitrogen content of malt is largely determined by the amount in the original barley. It has thus been found that the comparatively low percentages of nitrogen found in some superior malts are due to the fact that the latter are from barleys whose seeds were carefully selected, the plants being grown on sparsely manured soil to avoid excessive uptake of nitrogen. The results of the present experiment show that the quality attained after so much trouble and expense can be secured more easily and at much less cost by electrical treatment of the seeds previous to germination. The nitrogen content of the malt was reduced to such an extent as would have been possible only by starting with a barley of quality superior to that used in the experiment. Barley responds readily to nitrogenous manures; but since a grain of high nitrogen content is obtained together with the increased yield, the farmer is often obliged to sacrifice higher yield to attain better quality. The present observations show that by suitable manuring on the field followed by electrical treatment of seed, a high yield of grain as well as superior quality of malt can be secured.

Proteolytic enzymes of malt.—Recent investigations (Linderstrom-Lang and Sato, *Compt. rend. Trav. Lab. Carlsberg*, 1929, **17**, 17; Hopkins, *Biochem. J.*, 1929, **23**, 1022) have shown barley malt contains at least one protease and one peptidase. To determine whether the rapid movement of nitrogen observed in the case of electrically treated barley was due either to the activation, or increased production of the proteolytic enzymes, extracts from treated as well as untreated malts were prepared according to Mill and Linderstrom-Lang (*Compt. rend. Trav. Lab. Carlsberg*, 1928, **17**, 9) and their action on different substrates determined by the method of Linderstrom-Lang and Sato (*loc. cit.*). The results did not, however, bring out any difference between the activities of the enzymes from the two sources. The titration figures were almost identical at all stages, thereby suggesting that there was no appreciable increase in proteolytic activity as the result of electrical treatment.

Effect of electrical treatment on the germination of barley.—Some of the previous workers (*e.g.* Dunn, *loc. cit.*, Bennet, *loc. cit.*, Nehru, *Proc. Indian Sci. Congress*, 1930, 38) reporting on different methods of electrical treatment of seeds have stated that quick and efficient germination of seed together with rapid development of the seedling may be expected. To determine whether (*a*) similar advantages follow as the result of electrical treatment of barley under the optimum conditions, and (*b*) the increased enzyme activity observed during germination bears any relation to the development of the seedling, the

following experiment was made:—The seeds were steeped successively in water and electrolyte in the manner described already and a current of $4 \cdot 3 \times 10^{-3}$ amp. passed through them for 2 hours. They were then washed carefully and germinated on moist sand at a uniform range of temperature (23—25°). The rate of germination was determined by counting the number of seeds whose plumules had just emerged at each stage of observation. The development of plumules and radicles was studied by measuring at stated intervals the respective length in a large number of representative specimens. The data thus obtained were compared with those for seeds merely soaked in water and electrolyte respectively.

A study of the percentages of germination at different stages (Fig. VII) shows that the electrically treated seeds sprouted very much more quickly than the untreated. Up to the end of the third day, the treated seeds germinated at nearly twice the rate of the untreated; after that period, the latter began sprouting at a relatively higher rate than the former and although the percentages of their germination were, at no time, so high as in the case of treated seeds, the observations suggest that the effect of electrical treatment was essentially one of hastening germination. It is possible that the treatment helped the germination of a small percentage of seeds which would not otherwise have emerged, but the results of the present experiment show that such an effect is very much less pronounced than that of quickened germination of seeds which would otherwise have sprouted more slowly.

Further examination of the results of germination at various intervals in the different cases shows that about 50 per cent. of the total number of seeds germinated between the first and the fifth days and about 30 per cent. between the fifth and the sixth days. The effect of the electrical treatment appears to have been chiefly one of hastening the germination of the seeds that sprouted during the first of the two periods mentioned.

It is not possible to state from the above observations whether the early germinating seeds favoured by the electrical treatment were in any way different from the others. Since it is known that even among mature grains collected from the same ear, some germinate less readily than others, it is hardly likely that there could be any measurable physical or physico-chemical difference between the seeds that respond readily to the treatment and those that do not.

The results of the germination studies explain in part the increased diastatic activity observed in the case of electrically treated malts. The investigations of Brown and Morris and other earlier workers have shown that, depending on the prevailing climatic and other conditions

the diastatic power of germinating barley increases up to a certain period after which it decreases with considerable rapidity. There is also marked loss in the dry weight of malt owing to the major part of the solid matter having passed out into the seedlings. To secure the best results, the maltster is obliged to stop the germination of a batch of seeds on a particular day whether the germination of all individual grains is sufficiently advanced or not. The specimen of malt which is kilned at the desired point consists, therefore, of a certain percentage of seeds whose germination has proceeded up to the maximum diastatic power, and of seeds which have either over, or under germinated or not germinated at all. The diastatic power and the general quality of the malt will be determined more by the proportion of seeds which have germinated up to the desired point rather than by the others. The results of the foregoing trials lead to the inference that at the time of arresting germination on the fourth day, over 40 per cent. of the electrically treated seeds would have developed the maximum concentration of active enzyme, while in the case of the untreated the corresponding percentage should have been about 20. It thus follows that by effecting a quicker and more uniform germination, the electrical treatment helps to secure a malt with greater concentration of enzyme than would otherwise be possible. The increased diastatic power is also largely explained in this way.

The length measurements of plumules and radicles were carried out with seeds, whether treated or not, which germinated on the same day. The results may, therefore, be regarded as indicating the rates of development of the respective seedlings as distinct from the percentages of germination described in the previous experiment.

A study of the rates of development of the radicles (Fig. VIII) shows that those of the electrically treated barley grow about 50 per cent. more quickly than those of the untreated grain. In the case of the plumules, the difference is not appreciable up to the end of 48 hours, after which the treated grains develop much more quickly than the untreated, and maintain the difference up to the end of 120 hours. The correlation of development-rates of plumules and radicles between themselves, and with the simultaneous intake of nitrogen is positive, but not close. Thus whereas the intake of nitrogen within the first 20 hours is very rapid, there is no proportionate development of plumules and radicles. Between 48 and 72 hours the movement of nitrogen is very slow, but the development of the seedling proceeds rapidly during this period. A scrutiny of the results, suggests that there is a lag of about 24 hours between the intake of nitrogen and its visible effect on the development of the seedling. Such a period is presumably required for the movement of the nitrogenous compounds into the different parts of the plumules and radicles,

and their re-synthesis into proteins. The effect of the nitrogen intake is first seen in the case of the radicles, which respond almost immediately, while in that of the plumules it follows only about 20 hours later. Allowing for the lag in each case, it may be noted that there is striking agreement between the intake of nitrogen and the subsequent development of the seedling.

The foregoing observations suggest that in addition to increasing the efficiency of germination, the electrical treatment brings about quicker movement of nitrogen into the seedling followed by more rapid development of the plumules and radicles than in the case of the untreated grain. Since the better formation of the seedling will favour the conditions for rapid photosynthesis as well as assimilation of mineral nutrients from the soil, it may be inferred that the electrical treatment of the seed will prove beneficial in the early stages of plant life.

There is striking agreement between the diastatic activity of malt at different stages and the corresponding development of seedlings. The correlation is closer in the case of the plumules than in that of the radicles. Thus, it may be noted that up to the end of 48 hours the diastatic power of treated malt is not significantly different from that of the untreated, and the same applies to development of the plumules. After that period, however, the diastatic power of treated malt increases more rapidly than that of the untreated, and similarly the plumules of treated barley develop faster than those of the untreated grain.

In the early stages of plant life, the seedling is dependent on the parent seed for its nutrition. The movement of the food materials into the seedling is rendered possible by the activity of the different digestive ferments, particularly that of amylase on starch. Since the electrical treatment leads to the development of a greater concentration of diastase in the germinating grain, it may be inferred that there is quicker and more efficient conversion of starch into soluble carbohydrates in the case of the treated grain than in that of the untreated. Since the rapid movement and consequent assimilation of the nutrients connotes more favourable development of the seedlings, it follows that the better growth of the electrically treated seedlings is due to the greater activity of the different enzymes, particularly that of amylase, in the germinating seed.

In the mature plant system, the nitrogenous as well as the mineral nutrients collected from the soil proceed from the roots to the different parts of the plant, while the carbohydrates and other products of photosynthesis move in the reverse direction. A similar order of movement appears to prevail in the seedling. The correlation studies suggest

that the movement of nitrogen is first to the radicle and then to the plumule, while that of the carbohydrate is in the opposite direction.

Effect of electrical treatment of malting barley on the specific gravity and nitrogen content of wort.—Specimens of malts prepared from treated as well as untreated barley were ground to coarse powder, mixed with five times their weight of water and maintained at 55° for 6 hours. The mixtures were then boiled for 1 hour, allowed to cool, filtered, the solid washed and the filtrates made up to the same value in each case. The worts were compared for colour, aroma, nitrogen content and specific gravity. The experiments were repeated many times and the observations representing ranges of values have been presented in Table II.

TABLE II.

Source of extract: treatment of seed	Colour	Odour	Nitrogen per cent.	Specific gravity
Treated with $4{\cdot}3 \times 10^{-3}$ amp. for 2 hours ...	Orange ...	Highly aromatic ...	0·140–0·163	1·063–1·064
Untreated: Soaked in electrolyte ...	Light yellow.	Faintly aromatic ...	0·198–0·201	1·045–1·046
Untreated: Soaked in water	Pale yellow...	do.	0·207–0·233	1·042–1·045

It is rather difficult, at this stage, to explain the differences in colour and aroma though the starting materials and details of malting were the same in all the cases. The observations suggest that some caramelisation has occurred in the case of the treated specimen, but since the conditions of drying the malt and boiling the wort were identical, it is hardly probable that the differences observed were due to greater heating during those stages. Since the only difference between the various worts consisted in the original treatments of the barleys concerned, it may be inferred that the electrical treatment had produced an effect allied to kilning at a relatively high temperature. In this connection, it should be noted that strong heating as in the case of mild ale or porter malts tends to lower the diastatic power while the electrical treatment actually helps to increase it. The effects of the two treatments cannot therefore be regarded as identical.

The results of the replicate determinations of the nitrogen contents of the different preparations vary so considerably among themselves that it is not possible to draw any definite conclusion from them. It may, however, be said in a general way, that wort prepared from electrically treated barley contains less nitrogen than that from untreated grain.

The differences between the specific gravity of the different worts are more striking, that from treated barley corresponding to a malt extract of about 15·7 per cent. while those from untreated grain approximate to only 10·9 per cent. The maltose and dextrin contents vary in a similar manner and, reckoned according to the Brown and Morris formula, they would be 16·9 and 11·7 per cent. for the worts from treated and untreated barleys, respectively.

The foregoing observations raise various points of academic interest and practical importance. The deep colour and the sweet odour of the wort suggest that the electrical treatment had produced certain physico-chemical effects akin to that of strong heating without impairing the activity of the enzyme. The low nitrogen and the high sugar contents appear to be related and suggest that by the rapid removal of the nitrogenous compounds to the plumules and radicles, quick and efficient hydrolysis of starch and other polysaccharides by the different enzymes was rendered possible.

DISCUSSION.

The researches of Lintner, Osborne and other earlier workers have shown that vegetable amylases occur in association with proteins, chiefly albumins, from which it is not possible to separate them without impairing the enzymic activity. Sherman and Gettler observed that the distribution of nitrogen in a number of preparations of animal and vegetable amylases was the same as in proteins and concluded therefrom that the enzymes are proteins in nature (*J.A.C.S.*, 1913, **35**, 1790). Lüers (*Biochem. Z.*, 1920, **104**, 71) as well as Baker and Hulton (*J.C.S.*, 1922, **121**, 1929) obtained evidence to suggest that barley malt amylase is derived from the proteins of the grain. The observations of many of the later workers (e.g., Van Laer, *Z. Ges. Brauw.* 1914, **36**, 489; Willstätter, Waldschmidt-Leitz and Hesse, *Z. physiol. Chem.*, 1923, **126**, 143; Fricke and Kajá, *Ber.*, 1924, **57**, 765; Narayanamurti and Norris, *J. Indian Inst. Sci.*, 1928, **11A**, 134) did not support such views. No definite evidence has however been, so far, obtained either to establish the chemical nature of the enzyme or to exclude the need for its association with proteins.

It is generally known that malts derived from barleys rich in nitrogen possess high diastatic power (Rothamsted Conference on Malting barley, *loc. cit.*). The observations of Bishop (*loc. cit.*) have shown that the percentage distribution of nitrogen in barley is the same for all specimens belonging to a particular variety. It may be inferred from the above that the actual quantities of the different proteins present in the seed as well as in the derived malt would be proportional to the total nitrogen content of the former. Assuming

that barley malt amylase is a protein, it would follow that its quantity as well as its activity would depend on the nitrogen content of the seed. The results of the present investigation do not, however, lend support to such a view: the malt prepared from electrically treated barley contains less nitrogen but more active enzyme than the one from untreated grain. Since the lower nitrogen content of the electrically treated malt is due to the rapid movement of the former into the seedling, it would, therefore, appear that the amylase protein (if such it is) differs from the ordinary seed protein in that it cannot be hydrolysed by the proteolytic enzymes present in the seed and thus be transported to the seedling.

It has been estimated that during steeping, the barley grain absorbs about 50 per cent. of its weight of water (Ross-Mackenzie, Brewing and Malting, 1926) which causes the proteins and the starch to swell up to soft masses holding water and salts in their interstices. In such a condition the seed offers a low resistance to the passage of electricity and it may therefore be assumed that during the electrical treatment, quite a considerable part of the energy passed through the seed.

The barley grain is rich in albumins which are more sensitive to physical and physico-chemical changes than the other proteins and in view of the observations of Hardy, (*J. Physiol.*, 1899, **24**, 288) it may be inferred that even a current of relatively low density would have denatured quite a large part of the albumins and the other associated proteins of the seed.

The observations of Mendel and Lewis (*J. Biol. Chem.*, 1913–14, **16**, 55), Bateman (*J. Biol. Chem.*, 1916, **26**, 263), Finks and Johns (*Am. J. Physiol.*, 1921, **57**, 61) and later workers have established that proteins are digested by proteolytic enzymes very much more readily when denatured than if raw. It would therefore follow that the proteins of the electrically treated barley would be hydrolysed more readily than those of the untreated grain during germination. This explanation accounts for the quick transport of nitrogen into the seedlings of electrically treated barley, but casts considerable doubt on the possibility of malt amylase being a protein allied to albumins. It may be expected that a treatment leading to the denaturation of proteins would have inactivated, at least partly, an enzyme belonging to the same class of compounds: but the increased activity observed in the case of malt amylase shows that although the enzyme may occur associated with proteins, and may even have the same distribution of nitrogen as the seed protein, its structure and properties are different from those of proteins.

It is well known that malt derived from barley rich in nitrogen has a high diastatic power, but yields a wort of low specific gravity,

while the reverse is true of grain containing less of that element. Diastatic power being a measure of the initial rate of action of the enzyme on the substrate, it would appear from the above that the presence of nitrogenous compounds is favourable to the hydrolysis of starch by amylase in the early stages. The low gravity of the wort obtained after prolonged action of the enzyme suggests on the other hand that, in presence of excess of nitrogen, the conversion of starch into maltose and dextrins does not proceed to completion. The present investigation has already shown that (*a*) malt amylase acts independently of the nitrogenous compounds of the grain, and (*b*) rapid removal of nitrogen from the seed helps to increase the efficiency of the hydrolysis. The foregoing observations lead to the inference that although malt amylase occurs in association with the nitrogenous compounds of the grain, yet these, in some way hitherto obscure, hinder the prolonged action of the enzyme on starch.

In the initial stages of preparing a wort, much of the water-soluble as well as the salt-soluble proteins of the malt would pass into solution, the quantities thus peptised depending on (*a*) the quantities of inorganic salts present in the water, and (*b*) the total nitrogen of the grain. As the enzyme action progresses, the relatively high temperature, together with the increasingly acid reaction would bring about the denaturation and coagulation of the dissolved proteins, the quantities thus precipitated increasing at logarithmic rate as shown by Chick and Martin (*J. Physiol.*, 1910 **40**, 404; 1911, **43**, 1; 1912, **45**, 61, 261). The coagulated proteins would naturally settle on the particles of unattacked grist, the thickness of this protective layer depending on the quantity of protein originally in solution. At the same time, increasing quantities of hordein from incompletely malted grains would dissolve, the change in reaction facilitating their ready peptisation. Though hordein is not readily coagulated by heat, it may be expected that its occurrence around the unattacked particles of malt would add to the protective action exercised by the coagulated proteins.

Although the precise nature of the action of amylase on starch is still obscure, yet it is now definitely known that the hydrolysis is preceded by the adsorption of the enzyme on the surface of the starch gel. The occurrence of coagulated protein around the particles of unattacked grist would naturally interfere with the adsorption of the enzyme on the substrate, the extent of such hindrance depending on the thickness of the layer of protein. As already mentioned, the protein content of malt is proportional to its total nitrogen so that the extent of the hydrolysis of starch and, consequently, the specific gravity of the wort would be determined by the nitrogen content of the malt. It may, at the same time, be expected that some of the hordein and other resistant proteins which are not fully coagulated even on boiling the

wort would find their way to the fermenting vessel, where, with the greatly increased acidity, saturation with carbon dioxide, lowered surface tension and viscosity and decreasing specific gravity, they would pass into the suspensoid condition and thereby (*a*) prevent mechanically the active functioning of the yeast and (*b*) introduce cloudiness in the fermented liquid. The extent to which such effects are produced will depend on the nitrogen content of the original barley for reasons stated already.

The above explanation would probably account for the various observations recorded by previous workers on the significance of the nitrogen content of malting barley to the brewing industry. In view of the technical importance of the subject, it is desirable that a further systematic investigation on the relation of different types and conditions of vegetable proteins to the action of amylase on starch be undertaken. Although the presence of suspended protein is detrimental to the success of the fermentation, yet the observations of Chapman have shown that the occurrence of useful amounts of dissolved nitrogen in the wort is necessary for the nutrition of yeast and the successful fermentation (Rothamsted Conference, *loc. cit.*). In view of the above and the fact that the malt protease is not generally sufficiently active to hydrolyse the superfluous amounts of proteins, it would appear that the addition of small quantities of highly active and thermo-resistant vegetable proteases like papain to either the mash prior to boiling, or the wort during cooling, may help to achieve the desired end.

The available data do not throw any light on the varied effects of different (*a*) concentrations of electrolyte, (*b*) densities of current and (*c*) periods of treatment. Further study of the energy distribution between seed and electrolyte, and the attendant physical and physico-chemical changes should be made before the significance of such observations can be understood.

The conditions relating to the steeping of seed prior to the electrical treatment also deserve further study. Thus, an investigation of the effects of varying (*a*) the periods of steeping in water and electrolyte respectively and (*b*) the nature, the concentration and the reaction of the electrolyte, may lead to useful results. Owing, probably, to the prolonged preliminary steeping in water, or the quick movement of the absorbed nitrate into the seedling, none of the electrolyte could be detected in the malts prepared by us. It is, however, possible that under other conditions, considerable amounts of the electrolyte may be present in the seed, and thereby influence (*a*) the germination of the seed and the development of the seedling and (*b*) the quantity of the derived malt.

The results of the present investigation also have a practical interest. The importance of the different observations to the brewing industry has already been outlined. Similar advantages may be expected to follow in the manufacture of malt syrup, malt vinegar and industrial alcohol from starchy materials. It may, perhaps, be argued that electrically treated malt will have a low nutritive value owing to its reduced nitrogen content, and therefore be unsuitable for the manufacture of malted foods. Since, however, most of such food materials contain large quantities of evaporated milk or such other products which are rich in proteins, it is hardly likely that their nutritive value would thereby be appreciably impaired: on the other hand, the high concentration of amylase and sweet flavour would commend the use of electrically treated malt in such industries.

The possibility of the application of electrical treatment to the manufacture of active preparations of various digestive ferments also deserves systematic investigation. Although our preliminary observations indicated that castor-seed lipase may not respond to the treatment, it is yet possible that the amylases, lipases, proteases and other characteristic enzymes present in a large number of other seeds, or in plant and animal tissues, would be readily activated under conditions similar to those described in the present study.

The results of this investigation indicate that the electrical treatment of seed may prove beneficial in the early stages of plant life. In view, however, of the reports of Brenchley, Goddard and others (*loc. cit.*) regarding the ineffectiveness of the seeds treated by the Wolfryn process, it is desirable that further systematic field trials should be conducted. It is possible that factors such as age and previous condition of seed, season and soil conditions, may influence the efficacy of the electrical treatment of seeds. Further laboratory work should also be pursued along lines already suggested for malting barley, so that the maximum benefit may be derived at minimum cost; this has been begun in the biochemical laboratories of the Indian Institute of Science; but in view of the variety in (*a*) soil and climatic conditions of different countries, and (*b*) technical details relating to malting and related industrial operations, it is desirable that similar investigations should be conducted concurrently in other parts of the world.

SUMMARY.

The effects of (*a*) concentration of electrolyte, (*b*) current density, and (*c*) period of treatment of barley on the diastatic power of malt prepared from it were studied. It was found that preliminary soaking in water for 24 hours followed by steeping in a 0·1 per cent. solution of sodium nitrate and passage of alternating current (25 cycles, 210 V.,

and density, $4{\cdot}3 \times 10^{-3}$ amp.) for 2 hours led to over 100 per cent. increase in the diastatic power of malt.

A study of the various factors leading to the activation showed that (*a*) the activity of the enzyme unit was the same as in malt prepared from untreated barley, (*b*) no evidence of any change in either the properties of barley starch or the nature of enzyme activators could be obtained, (*c*) under the experimental conditions, even traces of the electrolyte could not be detected in the treated malt and (*d*) evidence was obtained to show that the concentration of the enzyme as well as of its liquefying and saccharifying components was greater in the treated malt than in the untreated.

The movement of nitrogen into the seedling was more rapid in the case of electrically treated barley than in the untreated grain. There was, however, no evidence to show that the protease activity of the former was greater than that of the latter.

The treated grains germinated in greater proportion and with more uniformity than the untreated. The seedlings of the former also developed faster than those of the latter.

The wort from electrified malt had a deeper colour, better aroma, less nitrogen and about 50 per cent. more sugar than the preparation from untreated malt.

There is evidence to show that although the enzyme amylase occurs in association with the proteins of the grain, it is not itself a protein. The observations suggest that, when present in excess, the proteins of the malt form protective layers around the starch and thus hinder the action of amylase from proceeding to completion.

The academic as well as the practical significance of the foregoing and allied observations have been discussed and future lines of work indicated.

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THE ORIGIN AND NATURE OF THE PEATY SOILS OF TRAVANCORE.

By T. R. Narayana Pillai and V. Subrahmanyan.

The *Kari* soils occur in patches among the taluks of Ambalapuzhay, Sherthalay and Vaikom of Central Travancore, and cover an aggregate area of over 20,000 acres. They are essentially dark and strongly smelling tracts lying below the sea level, subject to inundation by fresh water during the monsoon months (June–October) and by sea-water during the remaining part of the year. The *Kari* lands of Ambalapuzhay have been partially reclaimed during recent years and are chiefly under paddy cultivation, but the crops often fail partly owing to unfavourable climatic conditions (Pillai and Subrahmanyan, *J. Indian Inst. Sci.*, 1930, **13A**, 1) and partly owing to inadequate drainage, accumulation of decaying vegetation, intense acidity and increasing amounts of chlorides and sulphates. In view of the partial submergence of these lands by sea-water and the etiolation and 'yellowing' of rice plants so frequently observed about a month after sowing, Narayana Aiyar was led to conclude that the soils contain considerable quantities of the chloride and the sulphate of sodium, which inhibit the absorption of requisite amounts of the essential mineral nutrients as well as iron by the plants (Travancore Agric. Dept., 1923, Leaflet No. 126). He therefore suggested a system of drainage and liming for the amelioration of these lands.

Owing to the reasons stated above and their general inaccessibility, the major part of the *Kari* lands still remain wild and marshy regions with scarcely any vegetation except a coarse aquatic grass, the *Kathira*, which alone appears to flourish on it. The botanical identity of *Kathira* is still obscure. The grass is characterised by its small, submerged stem, profuse system of roots and prolific tillering: its leaves are hollow and rod-like, with thin, white internodes. It is used locally as a fodder.

There is no reliable record with regard to the origin and the early history of the *Kari* soils. Folklore points to the identity of these regions with the *Kandawa Vana* of Mahabaratha and there is increasing evidence to show that the area was once covered by forest. ~~In different~~ parts of the *Kari* tracts, partially decomposed wood as well as stumps of huge forest trees are still to be found. Stout stems of such trees together with the adhering roots have also been dug out, from time to time, from depths of six feet and over. It is, however, hardly probable that the original forest would have flourished under the present soil

conditions of *Kari* lands. There is a suggestion that the region was once more elevated, and owing perhaps to an upheaval became depressed below sea-level. This view is supported by recent evidence that the *Kari* lands and surrounding areas of *Kuttanad* were once a part of the Arabian Sea (K. Parameswaran Pillai, *Dept. of Agr. Travancore, Bull.*, 1924, 9). It may be presumed, from the above, that the lands were reclaimed from the sea partly by natural silting of rivers and partly by the efforts of man.

As already observed by Pillai and Subrahmanyan (*loc. cit.*), the *Kari* lands are characterised by the presence of (*a*) large quantities of partially decomposed organic matter and (*b*) abnormal amounts of soluble iron and aluminium salts. The physical properties and, to some extent, the chemical composition of the soils point to their being allied to the low-moor (Fen) peats (Waksman and Stevens, *Soil Sci.*, 1928, **26**, 113) of the temperate regions. The high temperature and the heavy rainfall of Travancore, the alternate inundation of the soils with fresh and salt water and the occurrence of considerable amounts of iron and aluminium salts suggest, however, that the conditions prevailing in the *Kari* lands are different from those in the low-moor peats. The present investigation was undertaken to determine (*a*) the conditions leading to formation of the *Kari* lands, (*b*) the relation between the organic matter of the soils and their vegetation, and (*c*) the agencies responsible for the solution of abnormal amounts of iron and aluminium.

EXPERIMENTAL.

The *Kari* soils examined in the present investigation were those from Ambalapuzhay. One of the authors (T.R.N.P.) toured the area and collected representative specimens to a depth of about 3 feet from different parts of the unreclaimed and the partially reclaimed tracts. It was found that the soil occurred in two distinct layers, the upper corresponding to the first foot, and the lower to the next 1½-2 feet. Specimens of the upper layer were more or less uniform; those of the sub-soil were more variable, being sometimes darker and more sticky, sometimes lighter and more sandy than those of the upper layer.

The mechanical composition, the available nutrients, the water-soluble constituents, the lime and other mineral requirements of the *Kari* soils have already been studied by Narayana Aiyar (*loc. cit.*) and by Pillai and Subrahmanyan (*loc. cit.*). To determine the nature and the extent of association between the mineral constituents of the soil and those of the existing vegetation, representative air-dried specimens of the soil and *Kathira* from different parts of the area were analysed by the A. O. A. C. (1925) methods. Table I presents the range of values thus obtained.

TABLE I.

Chemical constituent	Percentage			
	Kari soil		*Kathira*	
	From	To	From	To
Moisture	12·5	13·7	7·15	7·20
Loss on ignition...	38·0	45·0	78·7	80·5
Ash	41·5	48·0	12·1	14·1
Silica	26·5	32·2	5·3	7·1
Iron (Fe_2O_3)	3·5	4·0	1·3	1·5
Aluminium (Al_2O_3)	6·8	8·8		
Calcium (CaO)	0·6	0·8	0·7	1·1
Phosphoric acid (P_2O_5)	0·03	0·04	0·20	0·37
Potash (K_2O)	0·15	0·17	1·18	1·30
Sulphate (SO_4)	1·75	2·30	0·61	0·77
Chloride (Cl)	0·04	0·06	2·01	2·50

The moisture of the soil samples varied considerably with the period of air-drying. If the specimens were dried for only two or three days as in the case of ordinary soils, the moisture was in some cases as high as 30 per cent. On prolonged drying and storage the moisture diminished steadily, becoming constant at about 13 per cent., as observed above.

The mineral composition of the soil and its vegetation are not in any way related to each other. The distribution of soil-constituents points to a laterite origin, and shows a preponderance of iron, aluminium and sulphate over the others. The composition of *Kathira*, on the other hand, resembles those of the commoner flora of arable or grass-land except that its silica content is a little too low and the chloride somewhat high. Since the grass is aquatic and since the surface-water of the *Kari* regions contains fairly large quantities of chlorides, particularly when there are tidal rises, it would appear from the above that the grass is independent of the soil for its mineral nutrition. During the dry months of the year, however, the concentration of sulphates and of iron and aluminium salts in the surface water tends to increase, owing to continuous solution of those salts from the soil. Since such salts are toxic to the commoner forms of vegetation, the growth of *Kathira* under such conditions, together with the foregoing observations

regarding the distribution of its inorganic constituents, suggest that the grass is (*a*) tolerant of iron and aluminium salts in high concentration and (*b*) selective in its absorption of minerals.

It has already been mentioned that *Kathira* is used as a fodder, but only a part is so used; quite a considerable proportion of the *Kari* area is under deep water and hence inaccessible. The abundant grass in such tracts is not cut, but merely allowed to decay in the water-logged soil, and decomposition products of the grass would tend to accumulate. *Kathira* contains about 80 per cent. of organic matter and this accumulating in the soil would modify its composition. To ascertain the nature and the extent of the foregoing change a comparative study of the distribution of organic matter in the soil and in *Kathira* was conducted.

Distribution of organic matter.

In view of the indeterminate nature of the constituents affected by either fractional alkali extraction or partial oxidation, methods involving the use of such treatment were not attempted. Since the *Kari* soil contains considerable quantities of undecomposed plant residues and since *Kathira* is the principal form of vegetation, the nature of the association between the two was studied by comparing the amounts of the different plant constituents in each. The determinations were carried out on oven-dried specimens by the method of Waksman and Stevens (*loc. cit.*) and the results are shown in Table II.

TABLE II.

Fraction	Percentage			
	Kari soil		*Kathira*	
	From	To	From	To
Ether soluble	1·5	1·7	3·1	3·7
Alcohol (95 per cent.) soluble	0·8	0·9	1·2	1·3
Reducing sugars as glucose	*Nil*	*Nil*	2·5	3·0
Water soluble	2·3	2·5	10·2	14·6
HCl (2 per cent.) soluble	5·1	7·2	22·1	25·5
Pentosans as xylose	*Nil*	*Nil*	11·4	12·2
Celluloses	*Nil*	*Nil*	1·8	2·7
Lignin	21·7	30·5	27·5	34·5
Crude protein (N × 6·25)	3·2	3·5	5·6	6·1

The material extracted from the soil by (ethyl) ether was a dark-brown, sticky mass, about 7 per cent. of which dissolved in petroleum ether. The extract gave on evaporation a brown, oily liquid which had a peculiar and persistent aromatic smell and was readily inflammable. The liquid was soluble in alcohol, dilute alkali and concentrated sulphuric acid, but not in water or any of the dilute mineral acids. The yield of oil was too low to permit of systematic study. The petroleum ether extract of *Kathira* did not contain even traces of the oily liquid obtained from the soil.

To ascertain whether the oil arose from the woody material occurring in the soil, chips of the wood were freed from adhering earth, dried in the oven and extracted as before; on evaporating the extract an oily liquid with the properties described above was obtained. It may be inferred, therefore, that the oily liquid was derived from the woody material probably surviving from the previous forest age, and not from the present vegetation.

The residue from the petroleum ether extract was an amorphous, yellowish brown powder insoluble in water, but partially soluble in dilute alkali. It melted at 70-75° with some decomposition into a dark, viscous liquid which, on cooling, set to a glassy and brittle solid. The substance burnt with a smoky flame, and analysis showed it to contain carbon, hydrogen and sulphur, but no nitrogen. The composition and properties suggested that the substance was probably a mixture of resins. The same resinous substance formed the major part of the ether extract of *Kathira* while practically absent from the woody materials occurring in the soil. It thus follows that the ether-extractives of the soil were derived principally from *Kathira*, and from woody residues to a small extent only.

Russell (Soil conditions and plant growth, 1927, 178) observed that certain substances dissolving in volatile solvents and possessing wax-like properties tend to accumulate in soils rich in organic matter, and interfere with the wetting of the soil and the movement of water. The previous observations of the present authors (*loc. cit.*) have shown that the ether-extractives of the *Kari* lands form thin, oily coatings around the soil particles and are responsible for (*a*) the retention of high percentages of moisture for long periods and (*b*) the non-wetting of the dry soil even after prolonged standing with water. These and the present observations suggest that the wax-like constituents referred to by Russell are probably resins derived from undecomposed plant residues, tending to accumulate in soils which contain large amounts of organic matter and are not provided with adequate drainage or aeration.

It is a common observation on the *Kari* lands that dry weather within the first fortnight after broadcasting the seeds causes the

seedlings to wilt, though the soil, as a whole, may still contain a high percentage of moisture ; in many cases the wilting continues for several days even after showers. Probably the resins form thin, impenetrable coats around the soil particles and thus prevent the ready movement of water from within as well as from without.

With a view to estimating the extent to which the resins increase the moisture-holding capacity of the soil, a specimen (30 g.) of air-dried *Kari* soil was extracted with ether and while one portion (10 g.) of the residual soil was left in the resin-free condition, another (10 g.) was mixed thoroughly with the ether extract, and the solvent allowed to volatilise. The two specimens were then saturated with water, spread out to dry in air for two days, then dried in the oven and their moisture contents compared. It was observed that the specimen treated with resin contained 38·3 per cent. of moisture and that from which the resin had been extracted, 5·4 per cent. Thus the retention of high percentages of moisture by the *Kari* soil is due more to the minute quantities of resin than to the large amounts of other organic substances present in it. The previous observation of Pillai and Subrahmanyan (*loc. cit.*) that it is the nature of the organic matter rather than the total amount lost on ignition that determines the moisture-holding capacity of a soil, is also confirmed.

To obtain an idea of the extent to which the resins affect the solution of organic as well as inorganic substances from the soil, equal weights (20 g.) of air-dried and ether-extracted specimens respectively were treated with 50 c. c. portions of cold water and, after being shaken for 30 minutes, the extracts were passed through a Buchner filter with frequent washings until about 250 c. c. of the filtrate collected in each case. The soil sediments on the funnel were then washed repeatedly with hot water (60°) and the filtrates amounting to another 250 c. c. in each case collected. The different extracts, thus obtained, were evaporated to dryness on the water bath and their ash contents determined (Table III).

TABLE III.

Treatment of soil	Percentage			
	Cold water soluble		Hot water soluble	
	Loss on ignition	Ash	Loss on ignition	Ash
Air dried	1·95	3·93	0·35	0·33
Air dried and extracted with ether	2·15	4·33	0·50	0·53

The results show that in spite of the prolonged extraction, the soil containing the resinous matter still retains appreciable amounts of soluble substances. The retention will, no doubt, be very much more pronounced on the field, where the conditions for solution are much less favourable than in the above experiment so that the resins will interfere with the solution of organic as well as inorganic substances to a very much greater extent than that indicated by Table III.

The alcohol-soluble portions of the soil as well as *Kathira* resembled the corresponding ether extractives in their physical properties. They differed from the latter in elementary composition, containing nitrogen in addition to carbon, hydrogen and sulphur.

The foregoing observations indicate that *Kathira* is the chief form of organic matter undergoing decomposition in the *Kari* soil. The resins present in the grass tend to accumulate in the soil as they offer more resistance to decomposition than most other plant constituents. Absence of reducing sugars, pentosans and celluloses from the soil (vide Table II) suggests that these plant constituents undergo ready decomposition. The occurrence of small quantities of materials soluble in water and hydrochloric acid, respectively, indicates that (*a*) such substances undergo only partial decomposition in the soil, or (*b*) the water and acid soluble constituents of the soil are different from those of the decaying vegetation. The latter view is supported by the fact that the soil contains considerable quantities of soluble iron and aluminium salts. The presence of a high proportion of lignin in the soil indicates that this constituent offers considerable resistance to decomposition by the soil organisms. The nitrogen contents of the soil and the grass run in parallel, and allowing for the larger proportion of minerals in the former, it would follow that none of the plant nitrogen is lost from the soil by either leaching or denitrification.

From the above, it may be seen that the transformations in organic matter leading to the formation of the *Kari* soils are of the same type as those prevailing in the peaty soils of the temperate regions. The rapid decomposition of hemicelluloses and celluloses and the accumulation of lignins lend support to the general theory of Fischer and Schrader (cited from *Soil Sci.*, 1928, **26**, 117) regarding the origin of peat. The persistence of the ether-soluble constituents is not in accordance with the observations of Waksman and Stevens (*loc. cit.*) for low-moor peats, but is explained by the difference in climatic conditions and nature of the vegetation.

Distribution of Nitrogen.

This was determined to ascertain the nature and extent of the transformations undergone by the different forms of nitrogen present

in *Kathira* while decomposing in the *Kari* soil. The nitrogen contents of the different fractions were estimated by the method of Waksman and Stevens (*loc. cit.*) and that of free and saline ammonia by the method of Maclean and Robinson (*J. Agric. Sci.*, 1924, **14**, 548), the results being shown in Table IV.

TABLE IV.

Fraction	Percentage of nitrogen			
	Kari soil		*Kathira*	
	From	To	From	To
Ether-soluble	*Nil.*		*Nil.*	
Water-soluble	0·005	0·041	0·170	0·172
Free and saline NH_3	0·017		*Nil.*	
HCl (2 per cent.) soluble	0·061	0·061	0·302	0·351
H_2SO_4 (80 per cent.) soluble	0·047	0·050	0·177	0·190
Humin	0·382	0·401	0·310	0·321
Total (as calculated)	0·512	0·573	0·959	1·034
Total (as estimated)	0·540	0·572	0·999	1·071

The water-soluble fraction of the soil never contained more than 8 per cent. of the total nitrogen, but varied considerably within that limit. The corresponding figures for *Kathira* were very much higher, but did not vary appreciably with the specimen. The percentage of the acid-soluble fractions of the soil was much lower than that of the grass, suggesting that such fractions, together with the water-soluble forms, underwent ready decomposition in the *Kari* soil conditions. Ammonia was obviously one of the products of such decomposition, but the quantities present in that form do not account for more than 4 per cent. The percentage of humin nitrogen in the soil is higher than that of *Kathira*: this observation when considered together with the fact that the grass undergoing decomposition in the soil mixes with the large amounts of mineral matter which the latter contains, would suggest that the total amount of humin nitrogen in the soil very much exceeds that indicated by the percentage. Since the organic matter of the soil is derived chiefly from *Kathira*, it follows that the water and acid-soluble nitrogenous constituents of the grass are not lost from the soil, but are merely transformed into the more resistant humin. The above observations also help to account for those of Dachnowsk (*J.*

Agric. Res., 1924, **29**, 72) who noted that on continued submergence by water, the proteins of peat do not undergo ready ammonification. The accumulation of humin nitrogen in the soil does not, however, mean that it will ultimately become unavailable to plant nutrition: indeed, as observed by several workers in Europe and in America, one of the problems relating to a reclaimed peat soil is the prevention of the too rapid decomposition of organic matter and unaccountable loss of nitrogen which follows the introduction of drainage and application of lime. The difference between the quantities of total nitrogen shown by summation of the different forms, and that estimated experimentally, is not considerable. Allowing for the experimental error, it is hardly probable that anything more than traces of the soil or plant-nitrogen existed in forms other than those described above.

Distribution of minerals in the Kari soils.

It has already been observed that the *Kari* soils (*a*) are rich in calcium, iron and aluminium sulphates and other salts, but (*b*) contain only limited amounts of potassium chloride and other salts. Iron and aluminium salts are not, however, known to be present in such large quantities in any of the peats or peaty soils so far examined. In view of the above and the well-known toxic effects of high concentrations of soluble iron and aluminium salts on plant life, the extent to which the salts mentioned under (*a*) were present in the water-soluble form was determined and the quantities compared with those present in the ash (Table V).

TABLE V.

Description	Percentage in the unreclaimed area			
	Iron (Fe_2O_3)	Aluminium (Al_2O_3)	Calcium (CaO)	Sulphate (SO_4)
In ash	4·00	8·50	0·80	2·30
Water-soluble	0·10	0·50	0·54	2·73

The quantities of iron and aluminium salts extractable from the soil by cold water were less than 3 and 6 per cent. respectively of those present in the ash. The figures should not, however, be taken to represent quantities originally present in the soluble form, since the addition of large quantities of water would have (*a*) altered the reaction of the soil suspension and thereby reduced the solubility of the salts, and (*b*) brought about the hydrolysis of the iron and aluminium salts, resulting in the release of free acids and formation of sparingly soluble

basic salts. The above *inference was supported by the fact that the* water-extract contained free sulphuric acid (0·3–0·4 per cent.) and that its sulphate content (2·73 per cent.) was disproportionately higher than those of the associated metallic radicles. About 70 per cent. of the calcium present in the soil occurred in the soluble form: subsequent analysis showed that the latter consisted mostly of the sulphate, the quantity of surface water having been more than sufficient to dissolve it completely.

Considerable difficulty was experienced in determining the forms and mode of distribution of iron in the *Kari* soil. Owing to the instability of the ferrous salts and the readiness with which ferric as well as ferrous salts undergo hydrolysis, it was not possible to obtain any useful data from the analyses of cold and hot water extracts of air-dried specimens of the soils. Extraction with 1, 3, 5 and 7 per cent. solutions of hydrochloric, sulphuric and acetic acids respectively also led to no definite results. Estimation of iron in presence of soil organic matter also presented some difficulties. Titrating aliquot parts of soil extract against standard dichromate with diphenylamine as internal indicator was found to be unsatisfactory since the colour change was not sharp: with ferricyanide as external indicator, the end-point was partially masked by the colour of the extract. Direct titration against permanganate was unsatisfactory because of action by that reagent on the organic matter of the extract. A number of trials carried out with soil extracts to which known quantities of ferrous salts had been added showed, however, that in presence of acid and minute quantities of manganous sulphate the permanganate reacted first with the ferrous salt and then with the organic matter. The reaction with the ferrous salt was instantaneous while that with the humic matter was rather slow, so that the latter remained unaffected until all the ferrous salt had been oxidised to the ferric condition. Advantage was taken of this observation and by titrating with the ferricyanide as the external indicator it was possible to obtain fairly concordant results for soil extracts containing known quantities of ferrous salts. Ferric iron was estimated by the above method after reduction to the ferrous condition. For the latter purpose zinc was found to be more useful than stannous chloride. Analysis of many aqueous and acid extracts showed, however, that no ferrous iron was present in the air-dried soil and that the quantities of ferric salts present in the different specimens were small and variable. It would appear from the above that although, under the field conditions, *Kari* soils contain considerable quantities of ferrous iron in the water-soluble form, yet hydrolysis followed by air-drying converts them into basic ferric salts which are insoluble even in dilute mineral acids.

To trace the changes leading to solution of iron under field conditions, specimens of soil were submerged by varying quantities of water

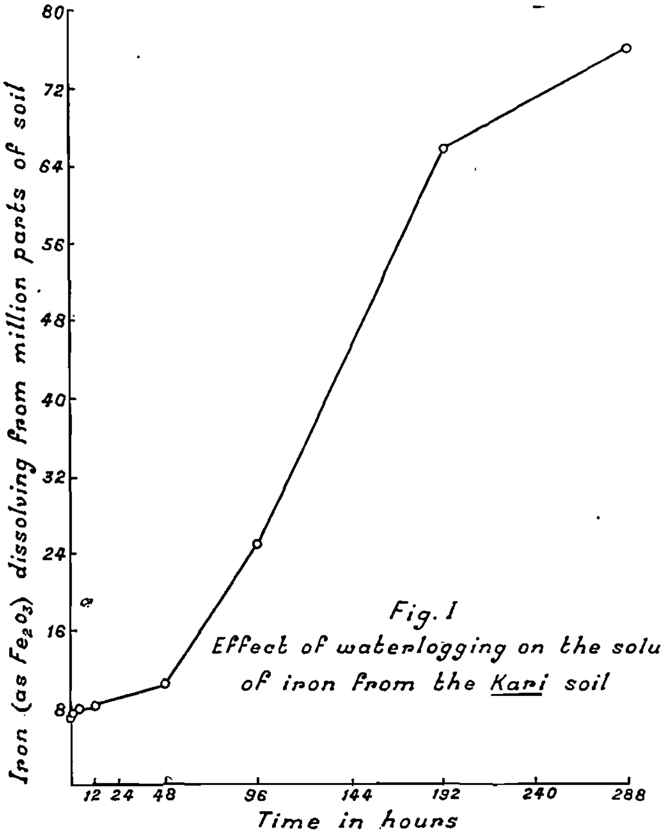

Fig. I
Effect of waterlogging on the solution of iron from the *Kari* soil

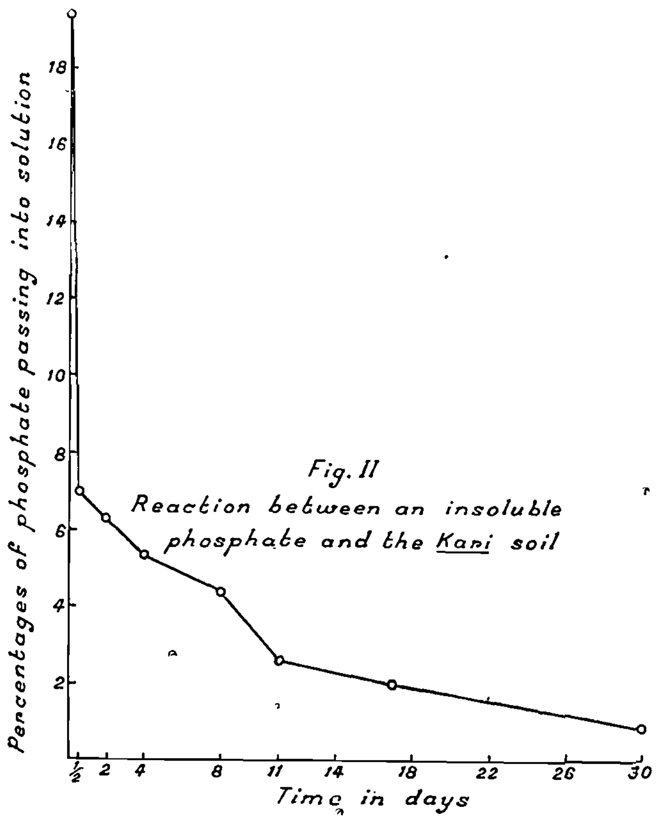

Fig. II
Reaction between an insoluble phosphate and the *Kari* soil

and the attendant reactions followed at 37° as well as at the room temperature (25–30°). It was noted in all the cases that the surface water became first acid owing to increasing quantities of sulphuric acid passing into solution. This was followed by evolution of minute quantities of hydrogen sulphide and solution of increasing amounts of ferrous iron. The reactions were more prominent in the case of specimens submerged by relatively small amounts of water (2 parts of water to 1 part of soil) and maintained at 37°, rather than in those standing under larger quantities of water or maintained at the lower temperature.

To estimate the iron dissolving under the field conditions, specimens (20 g.) of the soil were submerged by water (60 c.c.) at the laboratory temperature (25–30°) and the total quantities of iron passing into solution at different stages determined. The results (Fig. I) show that although only minute quantities of iron were present in the initial stages, increasing amounts passed into solution on continued standing under water. The rate of solution was highest between the fourth and the eighth days when quantities corresponding to over 4,000 parts per million of soil passed from the soil into the surface water. Solution of further quantities continued throughout the period of observation though the rate slackened, to some extent, after the eighth day. The above observations accord with those of Robinson (*Soil Sci.*, 1930, **30**, 197), who noted that considerable quantities of ferrous salts and sulphides were formed from submerged soils. Minute quantities of iron are necessary for plant development, but recent investigations have shown that high concentrations, particularly of ferrous salts, are toxic to plant life. The foregoing observations suggest that one cause of the crop failure so frequently experienced on the *Kari* lands, is the solution of iron in abnormal amount.

To determine the toxic action of dissolved salts on the rice plant the following experiment was conducted. The soil (1 part) was submerged by water (2 parts) in glass basins for 4 days when rice seedlings (0·5″–1″ high) were planted; after exposure to air and light it was observed that the seedlings soon became yellow, and within ten days were all dead or dying. Similar observations were made when the seeds were germinated in the *Kari* soil itself; after emergence of the first few leaves, the seedlings became yellow and died. The foregoing observations thus suggest that the yellowing of rice plants so commonly observed on the *Kari* lands is probably due to the presence of abnormal amounts of iron in the surface water. Simultaneously with the yellowing of the seedlings, it was observed that white, needle-like crystals formed incrustations at the sides of the germinating-basins; these were recrystallised from water and found to contain only iron, aluminium, calcium and sulphate.

To identify the principal inorganic salts in the surface water, this was decanted from the soil (200 g.), filtered through the Buchner and concentrated on the water bath. After 24 hours, minute crystals were rapidly filtered, and after washing and recrystallisation were found to consist exclusively of hydrated calcium sulphate. Alcohol of 95 per cent. was added to the mother liquor until precipitation was complete, the recrystallised material consisting solely of calcium sulphate. On allowing the alcohol-treated liquor to stand overnight, silky, fibrous crystals separated containing iron, aluminium and sulphate; they melted on gentle warming, but decomposed on strong heating emitting fumes of sulphur trioxide and leaving oxides of iron and aluminium. These crystals were, on further analysis, identified to be those of ferrous aluminium sulphate, $FeSO_4$, $Al_2(SO_4)_3$, $24H_2O$, a double salt occurring as the mineral, halotrichite, in Baluchistan, Persia and several other countries. Although known since 1802, Wirth first prepared it in the laboratory (*Z. angew. Chem.*, 1913, **26**, 81) by mixing equimolecular proportions of ferrous and aluminium sulphates in saturated solution out of contact with air. The spontaneous formation of this salt in the submerged *Kari* soils therefore supports the fact that (*a*) ferrous and aluminium sulphates are present in high concentrations in the soil and (*b*) the soil-conditions are anaerobic and hence unsuitable for air-loving plants and micro-organisms.

The investigations of Barber (cited from *Mem. Agric. Dept. India, Chem. Ser.*, 1913, **3**, 93) have shown that although the rice plant requires considerable quantities of water and grows best only under the conditions of the swamp soil, it is not really an aquatic plant. Its root-system resembles in structure and function those of the other cereals, thereby indicating that the plant requires adequate soil aeration. The anaerobic conditions prevailing in the submerged *Kari* soil coupled with high concentration of toxic ferrous and aluminium ions suggests that it is not suitable for the cultivation of rice.

It has already been shown that the total sulphur content of the *Kari* soil is higher than that indicated by the amount of sulphate present in the ash. The low value of the latter is explained by the presence of readily decomposable sulphates such as the ferrous aluminium double salt. The free acid released by hydrolysis on treating the soil with excess of water corresponded to 0·3–0·4 per cent. of sulphuric acid on the dry weight of the soil, but the estimates thus obtained could not indicate the quantities of the bases with which the acid had originally been associated. The total sulphur content of the soil as determined by Benedict's method (*J. Biol. Chem.*, 1909, **6**, 363) was 3·6 per cent. made up of 0·91 per cent. water-soluble sulphates, 0·19 and 0·22 per cent. respectively as sulphates soluble on shaking for 3 minutes with 5 and 7 per cent. hydrochloric acid, 0·29 per cent.

soluble sulphate as determined by Wiley's method (Principles and practice of agricultural analysis, Vol. I, 594): 1·4 per cent. was soluble in 2 per cent. alkali and 1·2 per cent. insoluble in either dilute acid or dilute alkali. Sulphides were absent from the air-dried specimens, but were readily formed on submergence in water.

The foregoing observations provide an outline of the distribution of sulphur in the soil, but do not throw much light on the forms in which it is present. In addition to the sulphates of sodium, calcium, iron and aluminium, the soil appears to contain sulphur in organic combination to the extent of about one-third of the total amount. The insoluble forms of sulphur may be partly organic and partly inorganic.

The high sulphur content of the *Kari* soils and the readiness with which the different forms undergo decomposition suggest that, under favourable conditions, the soils could be used in place of sulphuric acid for increasing the solubility of bones and mineral phosphates which are not otherwise readily available for plant nutrition. If all the combined sulphur is to undergo oxidation to sulphuric acid, or otherwise react directly with the insoluble phosphates, the concentration of active material would correspond to 10·8 per cent. of sulphuric acid on the dry weight of the soil. It would therefore appear possible to prepare a fertiliser similar to superphosphate by allowing suitable proportions of *Kari* soil and insoluble phosphates to either react together or undergo biological decomposition in presence of each other. The plant residues and the fairly large quantities of nitrogenous compounds present in the soil should facilitate the biological decomposition and increase the manurial value of the compost thus prepared. It is possible that the iron and the aluminium salts present in the soil would react with the dissolved phosphates and precipitate them, thereby reducing their solubility in solvents used for measuring the availability of phosphatic manures; but being in a fine state of division they would no doubt be readily available for plant nutrition.

To obtain a preliminary idea of the nature of the reaction between the *Kari* soil and an insoluble phosphate, the following experiment was made:—Small lots of the soil (25 g.) and calcium phosphate (0·355 g.) were weighed together into conical flasks and the mixtures treated with 25 c.c. of water; the suspensions remained at room temperature (25–30°) for varying periods and the water-soluble phosphates determined at different stages.

The results (Fig. II) show that water-soluble forms increased to about 20 per cent. in less than half an hour, but fell off steadily on further standing with the soil, water-solube phosphate being less than 1 per cent. at the end of 30 days. Since the *Kari* soils are rich in iron and aluminium salts which pass steadily into solution on continued standing with water, the above observations suggest that calcium

phosphate first dissolved by the sulphuric acid, and after reaction with the dissolved iron and aluminium salts precipitated as the insoluble phosphates of those elements. Precipitation in the soil need not, however, reduce the fertilising value of the phosphates, since they, being in finely divided condition, would still be readily available for plant nutrition. Indeed, a similar reaction is known to take place some time after adding either superphosphate or any of the other soluble forms to the soil. Under such conditions, although the availability of the phosphates, as determined by solution in dilute acids, may be low, the plants may still have abundant supplies of phosphates for their nutrition. In the above experiment, the availability at the end of 30 days as determined by Dyer's citric acid method (*J.C.S.*, 1894, **65**, 115) was 22·0 per cent. of the phosphate originally added. In view of the previous observation, it may be inferred that the quantity actually available for plant nutrition was considerably more than that indicated by the above figure.

Further experiments to determine the optimum conditions for the treatment of different insoluble phosphates so as to secure the maximum availability for plant nutrition are in progress and will form the subject of a later communication.

DISCUSSION.

From their geographical situation and seasonal conditions, it might appear that the *Kari* soils form a separate class. The results of the present investigation show, however, that the chemical and biological changes leading to the transformation of organic matter in the *Kari* soils are of the same type as those in the commoner peat soils. The accumulation of resins and ligniferous materials, retention of high percentages of moisture and conversion of the simpler forms of nitrogen into the more resistant humin are all characteristic of soils containing large amounts of organic matter without adequate drainage or aeration.

The *Kari* soils do, however, differ from peat soils in containing large amounts of (*a*) soluble iron and aluminium salts and (*b*) sulphur compounds, principally mineral sulphates. Whereas the occurrence of large amounts of organic matter is explained by the existence of the woody residues of the forest age and the decaying aquatic vegetation, the abnormal amount of the above-mentioned inorganic constituents is not easily explained. Though the West Coast soils are generally of laterite origin and are rich in iron and alumina, the conversion of those insoluble compounds into soluble forms, particularly such readily oxidisable compounds as ferrous aluminium sulphate, are features of the *Kari* soil which could be explained only by a careful biochemical study of the changes occurring therein.

It has already been observed by Pillai and Subrahmanyan (*loc. cit.*) that, during the monsoon months, a large part of the clay and the fine salt fractions of the single and double crop paddy lands respectively situated immediately above the *Kari* lands are washed down into the lower regions. Since the above-mentioned soil fractions are rich in iron and alumina, it may be inferred that considerable amounts of those minerals thus tend to accumulate in the low-lying areas. A part of the minerals would, no doubt, pass into the back-waters and be carried by the flowing tide into the sea: a part may also be mixed with the waters of the adjoining Vembanad lake: but a considerable portion would collect in the stagnant waters of the *Kari* land and settle in the soil on prolonged standing.

It has been shown by Subrahmanyan (*J. Agric. Sci.*, 1929, **19**, 627) that, in waterlogged soils containing large quantities of organic matter in readily decomposable forms, the dissolved oxygen of the surface water is rapidly engaged and considerable amounts of organic acids, chiefly lactic, acetic and butyric, are produced. A similar observation has been made by Subrahmanyan and Desikachar (unpublished data) in the course of their studies on the decomposition of plant-residues in waterlogged soils. It may be expected that the acids thus produced will react with the minerals of the soil and bring them, in increasing quantities, into solution in the surface water.

Gillespie (*Soil Sci.*, 1920, **9**, 199) has noted that on submerging a soil a reduction potential is set up. The observations of the present authors have shown that appreciable quantities of hydrogen sulphide are formed within the first few days after submergence of the *Kari* soils. Since the mineral sulphates were the chief sulphur-containing compounds present in the soils prior to their submergence, it may be inferred that the hydrogen sulphide was largely formed by the reduction of sulphuric acid released from the soils by hydrolysis. Under such conditions, it may be expected that (*a*) appreciable quantities of iron and aluminium would pass into solution and (*b*) ferric iron would be reduced to the ferrous condition. The different reactions described above may be represented by the following equations.—

(1) $A\ C_nH_{2n}O_n \rightarrow B\ CH_3\cdot CH(OH)\cdot COOH + C\ CH_3\cdot COOH + D\ CH_3\cdot (CH_2)_2\cdot COOH$ etc.

(carbohydrates of plant residues)

(2) $\left.\begin{matrix} Fe_2O_3 \\ Al_2O_3 \end{matrix}\right\} + 6\ HX \rightarrow FeX_3 + AlX_3$

(X represents the negative radicle of a monobasic organic acid)

(3) $FeX_3 + (H) \rightarrow FeX_2 + HX$

It has already been mentioned that the *Kari* lands are subject to inundation by sea-water whenever there are tidal rises. It may, therefore, be expected that the salts of the sea-water would react with the iron and aluminium salts rendered soluble by the biological reaction progressing in the soil. Since sea-water contains chiefly the chlorides and sulphates of sodium, magnesium and calcium and since the total amount of such salts brought in with every tide, would be proportionately much greater than the soluble iron and aluminium salts present in the soil, it would follow from the law of mass action that the major part of the iron and aluminium salts would be converted into the corresponding chlorides and sulphates. The chlorides are not, however, stable in presence of water, so it may be expected that they would undergo decomposition resulting in the formation of the corresponding metallic hydroxides and the release of hydrochloric acid, which would then be carried away by the flowing tide. This would account for the water above the *Kari* land containing chlorides and the consequent absorption of fairly large quantities of those salts by *Kathira*, which, as mentioned already, derives its nutrition exclusively from the surface water. Under the same conditions, the sulphates are relatively more stable. A part of them would combine with the organic matter of the soil and form floes from which they could not be readily leached out. In presence of excess of water, a part would undergo partial hydrolysis resulting in the formation of the corresponding stable, basic salts. It may, therefore, be expected that, after a succession of tidal ebbs and flows, the soils would contain only the sulphate of calcium, which is a sparingly soluble salt and the basic sulphates of iron and aluminium which are insoluble in water. On air-drying the soil taken at this stage, the ferrous salt would be readily oxidised to the ferric condition. The above explanation would account for (*a*) the leaching out of only calcium sulphate on first extraction with large amounts of water, (*b*) the absence of ferrous salts from both the aqueous and the acid extracts of the air-dried specimens and (*c*) the presence of only minute quantities of free sulphuric acid in aqueous extracts of the air-dried soil. The various reactions leading to the different transformations may be represented by the following equations.—

$$(4)\quad \left.\begin{matrix} FeX_2 \\ AlX_3 \end{matrix}\right\} + \begin{matrix} Na_2So_4 \\ NaCl \end{matrix} \longrightarrow \begin{matrix} FeSO_4,\ (FeCl_2) \\ Al_2\,(SO_4)_3,\ (AlCl_3) \end{matrix}$$

$$+\ NaX$$

(surface water)

$$(5)\quad \left.\begin{matrix} FeCl_2 \\ AlCl_3 \end{matrix}\right\} + H_2O \longrightarrow \begin{matrix} [Fe\,(OH)_2] \\ [Al\,(OH)_3] \end{matrix} \quad \begin{matrix} +\ HCl \\ \text{(Surface water)} \end{matrix}$$

$$(6)\quad \left.\begin{matrix} Al_2\,(SO_4)_3 \\ 2FeSO_4 \end{matrix}\right\} + 3H_2O \longrightarrow \begin{matrix} 2Al\,(OH)\,SO_4 \\ Fe\,(OH)_2,\ FeSO_4 \end{matrix} \quad \begin{matrix} +\ H_2SO_4 \\ \text{(Surface water)} \end{matrix}$$

(7) $\left.\begin{array}{l}[Fe(OH)_2],\ [Al(OH)_3]\\ Al(OH)SO_4,\ Fe(OH)_2,\ FeSO_4\end{array}\right\} + \xrightarrow[\text{(air-drying)}]{O}$

$$Fe_2O_3,\ Al_2O_3\ .$$
$$Fe(SO_4)_3,\ Al_2(SO_4)_3$$

(The above equations have not been balanced as the proportions are not known.)

On submerging the air-dried soils, ferric and aluminium sulphates would undergo hydrolysis resulting in the partial release of sulphuric acid and the precipitation of the corresponding basic sulphates. This would explain the presence of fairly large quantities of sulphuric acid, but only traces of iron in the aqueous extract of the soil. On further standing with water, biological decomposition of the plant residues would begin, resulting in anaerobic conditions and the development of organic acids (Subrahmanyan, *loc. cit.*, Subrahmanyan and Desikachar, *loc. cit.*). Increasing quantities of iron and aluminium salts would then pass into solution; ferric iron would be reduced to the ferrous condition. Basic salts of iron and the organic acids would be precipitated, while owing to the favourable reaction increasing quantities of ferrous and aluminium sulphates would be brought into solution.

High concentration of the two sulphates together with the favourable anaerobic conditions would have facilitated the formation of the relatively more stable double salt, as observed already. The different reactions leading to the formation of the ferrous aluminium alum may be represented by the following equations.—

(8) $\left.\begin{array}{l}Fe_2(SO_4)_3\\ Al_2(SO_4)_3\end{array}\right\} + H_2O \longrightarrow \begin{array}{l}Fe(OH)SO_4\\ Al(OH)SO_4\end{array} + H_2SO_4$

(9) $\left.\begin{array}{l}Fe(OH)SO_4\\ Al(OH)SO_4\end{array}\right\} + HX + H_2O \longrightarrow$

$$Fe_2(SO_4)_3\ Fe(OH)X_2$$
$$Al_2(SO_4)_3 + Al(OH)X_2$$

(10) $Fe_2(SO_4)_3 + (2H) \longrightarrow 2FeSO_4 + H_2SO_4$

(11) $FeSO_4 + Al_2(SO_4) + 24H_2O \longrightarrow FeSO_4\ Al_2(SO_4)_3\ 24H_2O$
(concentrated solution)

(Some of the above equations have not been balanced as the proportions of the products are not known.)

The foregoing reactions were further confirmed by identifying the more important intermediate products on repeating the natural

conditions in the laboratory. The organic acids were identified in the manner described by Subrahmanyan (*loc. cit.*). The solution of iron and aluminium salts as well as the reduction of ferric iron to the ferrous condition were also verified. Basic iron salts, largely in the ferric condition, were found to be present either as scum on the surface water or as rings at the sides of the flasks.

The possibilities of utilising the free acid formed from the *Kari* soil for rendering insoluble phosphates available for plant nutrition have already been indicated. That would appear to be a more satisfactory method of utilising the soil than that of burning it as fuel as is usually done in Europe and elsewhere. Although the *Kari* soil burns with a dull flame after prolonged heating, its high moisture and relatively low organic matter contents would detract considerably from its fuel value. Moreover, the acid fumes which will be evolved on heating the soil would poison the air and cause the rapid perishing of the materials which it is intended to heat.

SUMMARY.

The distribution of organic matter in the *Kari* soil suggests that the former is derived mainly from *Kathira*, an aquatic grass which flourishes in the surface water of the soil, and to a small extent, from the remains of a previous forest vegetation. As in other peat soils, lignins left over from the decaying vegetation form the bulk of the organic matter of the *Kari* soil. Nitrogen is present chiefly as humin: there is evidence to show that the soluble and the more readily decomposable plant-constituents are converted into that resistant form in the soil.

The high moisture contents of air-dried specimens of the soil have been shown to be due to the presence of resins which form protective coats around the soil particles and prevent the movement of water and plant-nutrients from within as well as from without.

The minerals present in the *Kari* soil, particularly iron and aluminium sulphates, do not appear to be related to the plant residues which make up its bulk. There is evidence to show that large quantities of iron and alumina are washed down into the *Kari* lands from the higher regions during the monsoon months, and that they are dissolved and further reacted upon in presence of organic acids formed by decomposition of plant materials and the sea salts brought in during the tidal rises. The mechanism of the biological and chemical reactions leading to the formation of the more important mineral constituents, particularly the characteristic ferrous aluminium sulphate [$FeSO_4\,Al_2(SO_4)_3\,24H_2O$], has been discussed.

The possibility of utilising the free sulphuric acid released from the *Kari* soil for rendering insoluble phosphates available for plant nutrition is indicated.

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THE RELATION BETWEEN SEEDS AND MICRO-ORGANISMS.

By T. R. Sathe and V. Subrahmanyan.

Although it is generally recognised that all seeds germinate under favourable conditions of temperature, moisture, and substrate, the various changes by which an apparently inert seed becomes a growing plant remain obscure. The early softening of the seed-coat, absorption of water and consequent swelling are essentially physical and can be readily followed : but the nature of the forces that link these with a succession of processes such as respiration of the seed, development of various enzymes and emergence of the seedling, forms one of the most fundamental and elusive problems in the study of plant-life.

The natural substrate for germination is the soil and the large number and variety of soil micro-flora led several of the earlier workers to suggest that the first stage in germination is induced by micro-organisms, which it was believed were responsible for the transformation of nutrients in the seed and the growth of the seedling. The study of the relation between the micro-organisms of the soil and the germinating seed or the growing plant has, however, been hindered by the fact that conditions suitable for vegetative growth are also favourable to the active functioning of microscopic life : proper conditions of temperature and moisture and the occurrence of useful amounts of decaying organic matter help the development of plants as well as micro-organisms and, in absence of any evidence to the contrary, it has been generally assumed that micro-organisms are necessary for the germination of seed and the development of seedlings.

From his studies on putrefactive microbes Duclaux inferred that micro-organisms are essential to germination; according to him, seeds sown in a soil devoid of microscopic life fail to germinate (*Compt. rend.*, 1885, **100**, 66). Owing to various technical difficulties which were subsequently realised, the observations of Duclaux could not be supported by later workers. Nilson (*J. A. C. S.*, 1904, **26**, 289) observed that lactic acid bacteria are invariably associated with germinating barley, and showed that the acid reacts with the seed proteins to produce enzymes, which convert the reserve food material into soluble forms required by the embryo. Nilson's observations were contradicted by Windisch and Schoenewold (*Woch. Brau.*, 1905, **22**, 200) who stated that barley can be completely sterilised by treatment with a solution of mercuric chloride in alcohol, without impairing the germinating capacity. Pickering (*J. Agric. Sci.*, 1907-8, **2**, 411)

observed that sterilised seeds of *Lilium italicum*, *Lilium perenne*, clover and spinach could be germinated on agar-agar jelly. Fowler and Sen found that acetone-producing organisms of the type of *B. Granulobacter pectinovorum* (E. P., 4845 of 1919) occurred in association with many cereals (*J. Indian Inst. Sci.*, 1921, **4**, 119). Fowler and Sreenivasaya noticed that a bacterium is always present on the seed of *Cassia tora*, used for reducing indigo in the indigenous dye-vat, and is responsible for the reduction of indigo to the soluble leuco-compound (*J. Indian Inst. Sci.*, 1921, **4**, 205). Fowler and Marsden concluded that an organism associated with coconut husk facilitates retting by loosening the fibre from the pectins and gums (*J. Indian Inst. Sci.*, 1924, **7**, 39). In view of the above and other observations concerning the occurrence of specific bacteria on the indigo-seed and inside the poppy-head, Fowler and Christie decided that there is a definite symbiotic or mutual relation between seeds and some of the micro-organisms associated with them (*J. Indian Inst. Sci.*, 1924, **7**, 253).

Other authors have adduced evidence that there is hereditary symbiosis between certain species of plants and some of the associated micro-organisms. Bernard observed a specific fungus to be necessary for germination of the orchid seed: the fungus was present on the growing plant and in the ripening seed (*Compt. rend.*, 1899, **128**, 1253). This observation was subsequently modified by Bernard himself and later contradicted by Kundson (*Bot. Gaz.*, 1922, **73**, 1) who germinated varieties of orchid in the absence of fungi. Freeman noted that *Lolium temulentum* is associated with a fungus carried from one generation to another through the seed (*Proc. Roy. Soc.*, 1902, **71**, 27). Miehe found that *Ardisia crispa* harbours an organism which is hereditary to the plant, but he did not succeed in separating the organism from the seed, so there is no definite proof that they exist in symbiosis (cited from *Zentralbl. Bakt.*, Abt. II, 1913, **37**, 142). Faber observed a similar phenomenon in the case of many *Rubiaceae* (*Jahr. Wissen. Bot.*, 1924, **54**, 243).

Symbiosis between two dissimilar forms of life would mean that they (*a*) are in some definite physiological association, (*b*) are, generally, mutually helpful and (*c*) cannot live or function normally without each other. A critical study of the various observations cited above will show that in no case has it been demonstrated that a specific organism or group of organisms is in symbiotic relationship with the associated seed.

Nilson's observations (*loc. cit.*) are not supported by sufficient biochemical evidence to show that the associated organism (*a*) penetrates the seed-coat and (*b*) produces lactic acid which reacts with the proteins of the embryo to form enzymes. The specific bacteria described by Fowler and some of his co-workers (*loc. cit.*) are really

those selected under the natural or artificial conditions favouring such organisms to the exclusion of most other forms of life: such observations cannot be inferred to throw any light on the relation between the original seed, or coconut, as the case may be, and the selected organism. The observation that a certain organism occurs in constant association with a seed does not necessarily mean that it is physiologically related, but indicates that the development and active functioning of both are favoured by similar natural conditions. Viewed in this light it appears doubtful whether any of the organisms claimed to be hereditary symbionts can be correctly thus described; many of them have not been isolated, so that one of the essential proofs of their symbiotic association is still lacking. Even Bernard's observations (*loc. cit.*), though of considerable interest, do not show whether the sterilised seeds were really in the right condition for germination. In the case of a thick-coated and slow-germinating seed like the orchid, proper ageing of the mature seed and prolonged steeping under favourable conditions are necessary to ensure germination; moreover, as appears from the present investigation, sufficient proof was not adduced to show that the antiseptic used for the sterilisation of the seed-coat was not the cause of suppressed germination.

In view of the inconclusive nature of the evidence so far obtained and the need for some definite information regarding the relation between seeds and micro-organisms in general, the present investigation was undertaken. The studies have been directed to ascertaining whether (*a*) there are specific organisms in definite physiological association with seeds and (*b*) micro-organisms either individually or totally have any influence, direct or indirect, on germination of the seed and the development of the seedling.

The following is an outline of the series of experiments conducted:—

(1) Systematic examination of seeds for micro-organisms occurring outside as well as inside the seed-coat. (2) Experiments to effect the sterilisation of the seed without injury to its vitality, and comparative study of various sterilising agents used for that purpose. (3) Germination of sterilised seeds under aseptic conditions as compared with that of unsterilised seeds under ordinary conditions, with special reference to time of germination and growth of seedling. (4) Response of seeds previously sterilised and then inoculated with the seed-organisms, or with different other forms of micro-flora, as compared with those of sterilised and untreated seeds.

Such seeds and micro-organisms as were found to be specifically associated with each other were tested to determine whether they satisfied the following conditions for symbiosis:—(*a*) Constant

occurrence of specific organisms in the seeds obtained from different samples. (*b*) If the symbiosis is absolute, the symbionts will fail to flourish without each other, i.e. the seed may fail to germinate, or the seedling may not thrive in the absence of the symbiont; similarly the symibotic organisms would fail to grow in the absence of the seed. (*c*) If the symbiosis is facultative, the symbionts will flourish more vigorously when in association than they would when separated from each other.

EXPERIMENTAL.

Search for specific bacteria.—Wherever possible, seeds were taken from fresh fruits under aseptic conditions in the laboratory and examined for bacteria harboured either on the seed-coat or in the body of the seeds. In cases where only seeds could be obtained, attempts were made to determine whether any particular organism was constantly associated with a number of representative specimens. The following seeds were examined :—*Citrus acida* (fresh fruit), *Ferronia elephantis* (fresh fruit), *Lycoperoicum esculentum* (fresh fruit), *Arachis hypogæa* (dry pods), *Cajanus indicus* (green pods), *Cocos nucifera* (whole fresh fruit from bazaar and local gardens), *Areca catechu* (whole fresh fruit), *Ardisia humilians* (from local Botanical Garden), *Ardisia crenulata* (from Penang, F.M.S.), *Hordeum vulgaris* (bazaar variety), *Triticum sativum* (bazaar variety), *Oryza sativa* (bazaar varieties), *Trigonella fenugræcum* (bazaar variety), *Pisum sativum* (green and dry pods), *Cassia tora* (bazaar variety), *Indigofera tinctoria* (from Bihar?), *Anona squamosa* (fresh fruit), *Gossipium herbaceum*, *Cannabis sativa* and *Ardisia crispa* (from Ceylon).

Before removing the seeds, the fruits were subjected to such sterilising treatment as they could tolerate without effect on the seeds. The lemon (*Citrus acida*), the tomato (*Lycoperoicum esculentum*) and ground-nut (*Arachis hypogæa*) were each dipped for 20 minutes in mercuric chloride solution (2 in 1000) and then passed through flame. The fruit was then punctured with a sterilised scalpel near a gas flame, the seed taken out with sterilised forceps and placed in petri-dishes containing nutrient agar and in tubes containing nutrient broth. The contents of some tubes were subsequently covered with layers of molten or liquid paraffin to facilitate the growth of such strict as well as facultative anaerobes as may be present. Several fruits (4–6) were examined in each case and from each fruit generally five cultures were made.

In the cases of wood-apple (*Ferronea elephantis*), coconut (*Cocos nucifera*) and arecanut (*Areca catechu*) more drastic methods of sterilisation were adopted. Thus the coconut was first dipped in mercuric chloride solution (2 in 1000) for 15 minutes, and then passed through flame. Holding the nut near the flame, a triangular cut was made in

the centre of a face with a sharp, sterilised scalpel. A few shreds of husk from the deeper layer removed by sterilised forceps were then plated and tubed in the manner described above; usually, three specimens were collected from each. Tender coconuts were given similar treatment, but the period of soaking in mercuric chloride was reduced to 10 minutes.

When a seed was examined for organisms inside the seed-coat or in the body of the seed, the surface was sterilised by steeping it in mercuric chloride (2 in 1000) for 10 to 15 minutes. The seed was then washed with sterile water, crushed in a sterilised mortar and transferred immediately to a petri-dish or a broth-tube. Control experiments were also made to determine whether the outside of the seeds had been completely sterilised. In the case of seeds obtained from the market, no attempt was made to study the flora on the seed-coat because it was expected that being exposed to dust, the organisms would be essentially those of the soil. Separation of the organisms inside the seeds was effected in the manner already described, the incubations being at 37°; the dishes and tubes were examined at frequent intervals.

The observations may be summarised as follows:—From over fifty inoculations made with each seed, growths were observed in three or four cases only, and the organisms isolated were not identical, the majority being the commoner forms of soil fungi, chiefly *Penicillia* and *Aspergilli* which appeared on the plates or in the tubes at the end of the first four or five days. Some of the growths were those of bacteria, chiefly of the spore-forming and spreading type such as *B. dendroides*, and were particularly prominent in the cases of *Cocos nucifera* (coconut) and *Areca catechu* (arecanut). They were also occasionally encountered in the seeds of *Gossipium herbaceum* (cotton) which, owing to the persistent adherence of fine fibres and the film of oil around the seed-coat, could not be properly wetted even after prolonged soaking in the sterilising solution. Control experiments revealed the same organisms in small numbers on the surface of seeds or fruits treated with sterilising agents in the manner described already.

A critical study of the results showed that the stray growths observed were merely accidental and probably due more to contamination from outside than to the presence of living organisms within. The heterogeneity of the isolated flora and the identity of most with the commoner soil organisms suggest that none of the bacteria or fungi was specific to the seeds or fruits concerned, and that they were all derived from the soil which might have settled on them as dust. The slow germination of the fungi or the spreading bacteria suggests that they were originally present as spores and thus remained unaffected even after prolonged treatment with antiseptics.

Although they cast doubt on the efficacy of what have been hitherto regarded as efficient methods of sterilisation, these observations do not preclude the possible occurrence of symbiotic organisms which could not be isolated by the foregoing methods; the specific organisms might not grow on artificial nutrient media, but might develop readily on their natural hosts, namely, the seeds themselves.

Attempts to isolate specific organisms.—The seeds examined were wheat, barley, paddy, beans (*Phaseolus vulgaris*), peas, *Cassia tora*, indigo, methi (*Trigonella fenugroecum*), cotton, *Ardisia humilians*, *A. crenulata* (Penang) and *A. crispa*. Fully developed and healthy specimens were chosen and after being treated in three different ways to remove the adhering foreign flora as far as possible, were inoculated into (*a*) sterile water and (*b*) sterile mashes (3 per cent.) of the different seeds. The treatment was (1) washing repeatedly with sterile water, (2) soaking in dilute copper sulphate solution (0·5 per cent.) for 3 minutes followed by washing with sterile water, and (3) treating with mercuric chloride (0·1 per cent.) for 2 minutes followed by washing with sterile water. After inoculation as described above, the tubes were incubated at 37° and examined at frequent intervals. They were also diluted with sterile saline and plated on (*a*) nutrient agar and (*b*) seed-mash agar.

The contents of all the tubes turned acid within a day or two after inoculation. The acids were identified as lactic, acetic and butyric, particularly the last, the quantities of which appeared to increase on standing. There was also noticeable evolution of gas at the end of the first few days. The tubes inoculated with seeds washed with sterile water showed the greatest activity and presented a larger variety of flora, particularly fungi, than the others. Microscopic examination did not show the preponderance of any particular organism or group of organisms in any case.

The growth on the plates confirmed the above observations. Fungi (*Penicillia* and *Aspergilli* and acid-producers were the next prominent organisms on plates prepared from diluted washings of seeds soaked in sterile water. Fungi were generally absent and acid-producers present only in small numbers on plates prepared from suspensions containing seeds previously washed with copper sulphate or mercuric chloride. Only in three cases were seen organisms other than those mentioned above. The plates made from cotton-seed were characterised by the occurrence of bacilli growing in rapidly spreading colonies and morphologically allied to *B. dendroides*: in the case of the coconut and arecanut bacteria similar to those described by Fowler and Marsden (*loc. cit.*) were also observed. Such organisms were always present on the fibres irrespective of locality or the stage of ripeness; they were absent from the respective kernel and

also from the milk in the case of the coconut. Some preliminary experiments also showed that in addition to the organism described by Fowler and Marsden many of the other organisms isolated from coconut and arecanut fibre could ferment sterile suspension of the husk more or less vigorously, thereby bringing about partial retting.

The foregoing observations appear to cast doubt on the possible association of the different fruits or seeds with specific organisms. The prominence of a certain organism or group of organisms under given experimental conditions would only indicate that the medium into which they were inoculated is favourable to their growth. Since in none of the experiments described above, no single organism was met with to the exclusion of others, it appears doubtful whether the study of any of those organisms would throw light on the possible response of the seed to the biochemical environment indicated by their presence.

Some experiments were conducted to determine whether the isolation of the more resistant bacteria such as those described by Weizmann (*loc. cit.*) and Fowler and Sen (*loc. cit.*) from cereals indicated any specific relation between those seeds and the allied organisms. The different seeds used in the previous trials were treated in the manner described by Weizmann, and the sub-culturing of the mixed flora first obtained was repeated at the boiling temperature of the mash until the flora finally obtained consisted only of organisms possessing identical morphological characteristics. The fermenting mashes were also examined for the production of gases, acidity, acetone and other allied products.

It was noted that in all cases the surviving organisms were rod-like, spore-forming bacteria similar to those described by Weizmann and others: the products of their activity were also the same. These observations combined with those of Fowler and Subrahmanyan (*J. Indian Inst. Sci.*, 1925, 8A, 71) who found acetone-producing bacteria in soil, sewage and various forms of vegetation, show that such organisms could not be regarded as exclusive associates of seeds. In view of the fact that the micro-flora on the surface of seeds, and those occurring in sewage are primarily those of the soil, it would appear that the thermo-resistant acetone-producing bacteria are normal inhabitants of the soil and not specific associates of seeds. The foregoing observations have thus shown that the different seed-treatments for the isolation of organisms suspected to be symbionts result only in partial sterilisation of the seed-coat and the selection of a few resistant species able to withstand the treatment.

Effect of sterilisation of seed-coat on the germination of seeds.—Although no direct evidence could be obtained to suggest the existence of any symbiotic or mutual relation between seeds and micro-organisms, it was still considered possible that the presence of the associated

organisms, either individually or as a group, might be necessary either to accelerate germination or to help in the development of the seedling. Some experiments were therefore carried out to determine the effect of partial or entire sterilisation of seed-coat by treatment with various antiseptics. Phenol, ethyl alcohol, formaldehyde, bleaching powder, copper sulphate and mercuric chloride were tried for the purpose and it was found that the last three were efficient, and at the same time the most convenient.

The seed-bed was made up of fine white sand prepared by boiling coarse sand with concentrated hydrochloric acid and then washing repeatedly to remove the last traces of acid. The sand was collected in glass basins with close fitting covers and conical beakers fitted with cotton wool plugs, and after being moistened, autoclaved intermittently at 10 lbs. and for 20 minutes until inoculation tests showed that the sand was completely free from living organisms.

Some preliminary tests were made on paddy, wheat and barley to determine the extent of sterilisation attained after treatment with dilute solutions of copper sulphate (1–2 per cent.) and mercuric chloride (0·1–0·2 per cent.), respectively. The grains in lots of 10 to 15 were treated with the antiseptic solutions for 5 minutes in each case. Representative specimens of the treated grains were shaken with sterile saline and plated out on nutrient agar in the usual way. On examining the plates on the fourth day it was found that the 0·2 per cent. solution of mercuric chloride had almost completely sterilised the seed-coats while solutions of lower strength were not so effective. Treatment with copper sulphate was still less satisfactory.

Following Wilson's method (*Amer. J. of Bot.*, 1915, **2**, 420) bleaching powder was tried for a similar purpose. From a commercial specimen of bleaching powder containing 25 per cent. available chlorine, aqueous solutions containing 2, 4 and 6 per cent. of available chlorine were prepared. The grains were treated as before and tested for the extent of the sterility of their seed-coats. It was observed that short-period treatments were not very effective and that soaking for about six hours in solutions containing 4 or 6 per cent. available chlorine was necessary completely to sterilise the seed-coats. It was however noted that the seeds thus treated did not germinate properly; the percentage of germination was low and the development of such seedlings as emerged was very poor. Treatment for even 8 hours with the solution containing 2 per cent. available chlorine was not effective in sterilising the seeds. In view of the weak sterilising action of copper sulphate and bleaching powder, and the possible unfavourable effect of prolonged treatment with such antiseptics, it was considered desirable to use mercuric chloride in subsequent experiments.

To determine the direct action of mercuric chloride adhering to the seeds in minute quantity representative specimens of the different grains were soaked in solutions of concentration ranging from 0·0025 to 0·2 per cent. for 10 minutes in each case, and allowed to germinate in sand-basins prepared as described. The percentage of germination, the general appearance of the seedling, and the development of shoot and root were determined in each case.

The observations showed that although all the seeds germinated at the end of three days, none of the seedlings developed properly. Those from seeds previously soaked in concentrations of 0·0025–0·05 per cent. suffered the least, but even they showed distinct weakness in root development within the first six days. After a week all the seedlings showed the effect of mercury poisoning in increasing degree. The experiments were therefore repeated washing the treated seeds with sterile water and comparing the growth of seedlings raised from them with those from untreated seeds. The observations are recorded in Table I.

TABLE I.

Concentration of mercuric chloride	Grain treated and washed	Grain treated and not washed
	Paddy	
1/1000	Seedling identical in appearance and growth with control	Roots not properly developed
2/1000	Apparently healthy roots; somewhat long	Roots short ; seedling unhealthy
	Barley	
1/1000	Identical with control	Roots slightly shorter than those of the control
2/1000	Resembles control ; roots not well developed	Poor root development
	Wheat	
1/1000	Similar to control ; bright in colour	Weak and unhealthy roots
2/1000	Root system elongated ; otherwise the same as control	Do.

The observations show that partial sterilisation of the seed-coat with a powerful antiseptic does not appreciably affect germination and the development of the seedling if the last traces of antiseptic are carefully removed. The above experiment was then repeated with

peas and tomato; the antiseptic was not so effective as with the cereals, but the germination results were similar to those described above.

It may be inferred from the foregoing observations that sterilisation of the seed-coat does not affect either the germination of the seed or the growth of the seedling if the last traces of antiseptic are removed. The seedling is highly sensitive to chemical poisons, and as in the case of copper sulphate or bleaching powder would show the effect thereof even if the seed-coat is only partially sterilised. It is not improbable that some of the earlier observations attributed to absence of associated micro-organisms were really due to minute quantities of antiseptic not completely removed after sterilising the seed-coat.

Further experiments were then made with the seeds of *Cassia tora*, *Trigonella fenugræcum*, *Phaseolus vulgaris* (French beans), tomato, peas, barley, wheat and paddy to ascertain whether addition of the mixed flora associated with the different seeds or soil extract to the sterilised seeds had any beneficial effect on the development of the respective seedlings. The seeds were sterilised by treatment with a 0·1 per cent. solution of mercuric chloride, and washed with sterile water to remove the antiseptic. They were then divided into three lots of which one was control, one treated with washings from the respective seeds and the third with suspensions of soil extract. The seeds were then spread out to germinate on sand-beds prepared in the manner described already. The rate of germination of the seed as well as the development of the seedling were determined in all the cases.

The results showed that the variations of treatment produced no visible effect on the germination of the different seeds: nor did the seedlings show any difference in the cases of paddy, *Cassia tora*, *Trigonella fenugræcum*, *Phaseolus vulgaris*, and peas. Some of the seedlings of barley, wheat and tomato appeared to be slightly benefited by suspensions of the mixed flora present on their respective seed-coats, but the observation could not be confirmed on repetition. The seedlings whether mixed with the associated flora or not, developed in a similar manner in all the cases. It was, therefore, inferred that (*a*) the associated bacteria have no influence on either germination or the development of the seedling, and (*b*) the better formation of seedlings observed in some cases was merely accidental and probably due more to the presence of certain readily available nutrients in the soil or seed extract than to the presence of micro-organisms.

Effect of inoculation of specific organisms on germination and growth of seedlings.—Having seen that no direct evidence of any

mutual relation between the seeds and associated micro-flora could be obtained, some attempts were made to determine whether active cultures of some of the commoner types of soil-organisms could appreciably influence germination.

Nilson (*loc. cit.*) observed that during the germination of barley, the lactic acid bacteria then prominent pass inside the hull of the grain. The previous observations of the present authors not having supported such a view, some experiments were conducted to test the above by more direct methods.

The seeds of *Dolichos lablab*, peas, barley, wheat and paddy were steeped in water, salt solution (1 per cent.), sulphuric acid (0·001 *N*) and sodium carbonate (0·5 per cent.) respectively for varying lengths of time (30 minutes–6 hours) to soften the seed-coats, and then spread out on sand-beds (unsterilised) to germinate. Representative specimens were removed at convenient intervals, fresh sections prepared, fixed, stained with methylene blue and examined for possible penetration by micro-organisms, but in no case could any living organism be observed.

In view of the possibility of the penetrating organisms not being present in sufficient numbers, some further experiments were made by adding aqueous suspensions of representative soil-organisms to various seeds soaked in different ways as described. The organisms used for inoculation were, *B. coli*, *B. lactis aerogenes*, *Hansenia apiculata*, a wild yeast originally found associated with rice, *Aspergillus niger*, and *Actinomyces Scabies*. The seeds were inoculated by soaking them momentarily in aqueous suspensions of the different organisms, and then spread out on sand to germinate. Representative specimens were removed at convenient invervals, washed with alcohol and sectioned; the sections were fixed, stained and examined for penetration.

It was observed that although the organisms persisted on the outer coats they had not penetrated the seed, even in a single instance. The seeds germinated at about the same rate and in the same proportion as those untreated. The growth of seedlings also appeared to be unaffected by the inoculated organisms. A few seedlings that had been moistened too freely were partly overgrown by the vegetative mycelia of *Aspergillus niger*, but were otherwise unaffected. Since however such fungus growths appear occasionally even on normally germinating seeds, if wetted profusely, it may be inferred that the overgrowth of the fungus was merely accidental and caused primarily by faulty watering. It follows that micro-organisms do not penetrate the seed and cannot therefore directly influence germination. The foregoing observations do not, however, preclude the possibility of products formed by the activity of the inoculated organisms passing

into the seed and thereby affecting, indirectly, the development of the seedling.

Is the presence of micro-organisms necessary for the production of lactic acid in the germinating seed?—Nilson (*loc. cit.*) showed that lactic acid is present inside the germinating seed. In view of the previous observations it appeared probable that minute quantities of acid produced outside the seed-coat by different organisms might have passed into the seed and thus helped the germination. Some experiments were therefore made to determine whether lactic acid is present in germinating seeds and, if so, whether micro-organisms are necessary to produce it.

Healthy seeds of paddy, wheat, barley and peas were selected and one half sown on a sand-bed, the other half being sterilised with mercuric chloride as described already and then sown. Representative specimens of the seeds were taken at different stages before and after germination and examined for the presence of lactic acid. The wet seeds were ground to paste, treated with dilute sulphuric acid, dried on the water-bath and extracted in four soxhlets with ether. After distilling off the ether, the extracts were taken up with water and tested for lactic acid.

It was observed that all the seeds whether sterilised or not, gave positive Uffelmann and thiophene tests for lactic acid at different stages in the course of their germination. In the case of wheat the acid was found to be formed within 8 hours after sowing, while in the case of barley and paddy the minimum periods required for acid formation were 30 and 60 hours, respectively; the reaction was less pronounced with peas. The small amount of acid precluded quantitative determinations being made by any of the known methods.

It may be inferred from the above that (*a*) lactic acid is formed in minute quantities in all germinating seeds and (*b*) the acid is produced by the seed itself and not by micro-organisms inside the seed-coat. The function of lactic acid in the germinating seed has not received much attention; it is probable that, as in the case of higher organisms, it is a product of carbohydrate metabolism and associated with respiration.

Effect of chemical treatment on germination and the activity of associated micro-organisms.—Although the previous observations had shown that the seed and the associated micro-flora are physiologically independent, it still appeared probable that they might exist in mutual relation under certain abnormal conditions. To test the above, the seeds of *Dolichos lablab*, paddy, barley and wheat were sown in sand-beds and treated with (*a*) antiseptics (thymol and phenol), (*b*) a bacterial food (glucose), (*c*) a product of bacterial metabolism (lactic acid), (*d*) a plant nutrient (Knop's food solution), (*e*) an inert salt (sodium

chloride) and (*f*) distilled water (control). Finely powdered thymol (0·05 per cent.) mixed uniformly with the sand; phenol (0·05 and 0·025 per cent.), lactic acid and glucose (0·05 per cent.) were used in aqueous solution.

Knop's food solution was prepared by dissolving 0·25 g. of magnesium sulphate, 1 g. of calcium nitrate, 0·15 g. of dihydrogen potassium phosphate, 0·12 g. of potassium chloride and a trace of ferric chloride in 1 litre of water, and added in quantity sufficient to moisten the seeds; sodium chloride was added in a 0·05 per cent. solution. The moisture content of the sand-bed was maintained constant by adding distilled water from day to day. The root-length and shoot-height of seedlings as also the numbers of bacteria adhering to seeds were determined at the end of six days. The observations have been summarised in Tables IIa, IIb, IIc and IId.

TABLE IIa.

PADDY

Seed treated with	Average root length in mm.	Average shoot length in mm.	Bacteria per seed (average) in thousands	Remarks
Distilled water	26·7	55·3	73	Seedlings normal (control)
Thymol	No germination	...	18	No visible effect on the seed-coat
Sodium chloride	22·7	48·3	59	Seedlings healthy, but not well developed
Glucose	23·7	49·0	Uncountably large. Profuse growth of fungi.	Seedlings healthy, but somewhat pale
Lactic acid	Poor. Overgrown with fungi	Poor. Overgrown with fungi	Uncountably large. Profuse growth of fungi	Seedlings started healthy—growth checked after 3 days—Warped root development
Knop's nutrient	34·0	77·3	97	Seedlings bright and healthy—rapid development
Phenol (0·05 per cent.)	No germination	...	7	Seed-coat turned dark brown

TABLE IIb.

BARLEY

Seed treated with	Average root length in mm.	Average shoot length in mm.	Bacteria per seed (average) in thousands	Remarks
Distilled water	40·0	71·0	71	Development of seedling normal (control)
Thymol	No germination	...	21	No visible effect on seed-coat
Sodium chloride	35·3	61·7	64	Seedlings normal
Glucose	33·7	57·3	Uncountable. Spreading colonies	Seedlings not well developed
Lactic acid	12·7 Partly over-grown with fungi	36·3 Partly over-grown with fungi	Uncountable. Overgrown with fungi	Growth of seedlings suppressed after the first few days—root development poor
Knop's nutrient	50·6	100·0	115 Largely over-grown with fungi	Seedlings healthy and well formed
Phenol (0·05 per cent.)	No germination	...	11	Seed-coat stained brown

TABLE IIc.

WHEAT

Seed treated with	Average root length in mm.	Average shoot length in mm.	Bacteria per seed (average) in thousands	Remarks
Distilled water	40·0	58·3	69	Seedlings normal (control)
Thymol	No germination	...	14	No germination even after 12 days
Sodium chloride	38·0	59·0	54	Seedlings healthy—indistinguishable from control

TABLE IIc.—(*contd.*)

WHEAT

Seed treated with	Average root length in mm.	Average shoot length in mm.	Bacteria per seed (average) in thousands	Remarks
Glucose	32·3	49·0	Uncountable. Plates overgrown with fungi	Seedlings unhealthy
Lactic acid	9·0	31·0	Overgrown with fungi	Seedlings developed normally during the first 4 days; roots warped after that date
Knop's nutrient	45·0	87·0	84	Seedlings healthy and well developed
Phenol (0·05 per cent.)	No germination	...	7	Seed-coat stained black

TABLE IId.

Dolichos lablab

Seed treated with	Average root length in mm.	Average shoot length in mm.	Bacteria per seed (average) in thousands	Remarks.
Distilled water	42·7	75·6	85	Seedlings normal (control)
Thymol	No germination	...	23	Only one seed germinated
Sodium chloride	42·7	63·0	69	Seedlings apparently healthy
Glucose	41·3	63·3	Uncountable. Profuse growth of fungi	Seedlings pale and unhealthy
Lactic acid	Overgrown with fungi	Overgrown with fungi	Profuse growth of fungi	After the shoot was about 20 mm. in length growth ceased

TABLE IId.—(*contd.*)

Dolichos lablab

Seed treated with	Average root length in mm.	Average shoot length in mm.	Bacteria per seed (average) in thousands	Remarks
Knop's nutrient	64·7	100·7	77	Seedlings healthy and well developed
Phenol (0·05 per cent.)	No germination	...	11	Most of the seeds blackened

It was observed also that the roots of seedlings raised on lactic acid tended to turn away from the seed-bed, thereby indicating that the acid was toxic. The roots tended to turn upward rather than go deeper into the sand-bed, so that the seedlings showed signs of being starved for want of sufficient nutrition. Profuse growths of fungi (chiefly *Penicillia*) were noticeable around seeds treated with glucose and lactic acid. Seeds treated with Knop's nutrient were generally clean and free from visible growths of fungi though the plates prepared from them contained numerous colonies of fungi in addition to those of the bacteria. Seedlings raised on glucose were invariably overgrown with fungi which covered the roots as well as the shoots and thus interfered with the normal development of the seedlings. Thymol had less inhibiting action on the bacteria than phenol, but was equally effective in checking the germination of the seed. Owing perhaps to the low concentration, sodium chloride was without much effect on either seedlings or bacteria : from the present results, its action would appear to be one of slight depression on the growth of seedlings as well as the multiplication of bacteria.

The foregoing observations indicate that seeds and associated micro-organisms do not always respond in the same manner to chemical treatment. In presence of a readily decomposable carbohydrate like glucose, bacteria and fungi increase at rapid rates while the growth of the seedling is actually suppressed. Lactic acid is toxic to the seedling, but encourages the spread of fungi. Knop's solution is beneficial to the seedlings but the associated micro-organisms do not respond so readily to it as they do to lactic acid or glucose ; there is marked increase in bacterial numbers in that nutrient, but the growth is very much less than in glucose. Being essentially saphrophytes, these organisms are naturally more favoured by a nutrient containing readily available carbon than by one which is made up of only minerals. The micro-organism numbers are slightly reduced and the development of seedlings appreciably retarded by

treatment with sodium chloride. Phenol and thymol are both toxic to bacteria and inhibit germination. Since similar toxic effects have been noted in the case of the micro-organisms even when they occur independently of the seeds, it may be inferred that the above observations cannot be attributed to any mutual relation between those two forms of life.

To determine whether the abnormal effects noticed in the case of seedlings treated with lactic acid were due to the high concentration, some experiments in treating the seeds of *Dolichos lablab*, paddy, wheat and barley with lactic acid solutions of lower concentrations (0·0025, 0·001 and 0·0005 per cent.) were made, germination and development of the seedlings being studied in the manner described already. It was observed that although the seeds germinated readily, the growth of the seedlings was rapidly affected as in the previous experiment. Root development was greatly retarded and, after the first four days, growth was almost negligible. The effect was most pronounced in the case of seeds treated with 0·0025 per cent. solution of the acid.

Some experiments were also conducted to determine whether the inhibitive action of thymol on germination was due to its chemical nature, or to its germicidal action in checking bacterial growth on the germination beds. The seeds were sown on beds treated with thymol-water (water filtered from finely powdered crystals) and the moisture content maintained by sprinkling the beds with the same extract once in twenty-four hours. Rates of germination as well as bacterial counts were determined in each case. It was observed that all the grains on the thymol-water beds germinated in the normal way, thereby indicating that low concentrations of that antiseptic were harmless. The bacterial counts on the other hand were about the same as in the previous experiment. This observation lends further support to the inference that germination is unconnected with bacterial activity.

Similar experiments with seeds treated with toluenated water showed that although the bacterial numbers were not appreciably affected, the seeds did not germinate at all. Addition of seed or soil extracts to the treated seeds did not lead to any improvement, thereby showing that toluene is deadly to the embryo and its action is not influenced by the presence or absence of micro-organisms. This observation also shows that the seeds and the associated bacteria are really independent of each other and respond differently to the same treatment.

DISCUSSION.

The results of the present investigation do not support the existence of any symbiotic, mutual, or other physiological relation between seeds and associated micro-organisms. The two forms of life,

one dormant and the other active, occur only in physical association and, under normal conditions, function independently of each other. They do generally respond in the same manner to a variety of physical and physico-chemical conditions, but do not, on that account, develop any physiological association with each other. Under some conditions, one form may be favoured almost to the suppression of the other; under others, the seed and associated bacteria may be both destroyed.

The earlier part of the work has shown that the normal micro-flora of the seed are essentially those derived from the soil. The dust which covers the seed-coat is only finely divided soil and, naturally, contains all the commoner soil-flora. All attempts to isolate the hypothetical specific associates have resulted only in selection of one or more of soil-organisms as could withstand the treatment given. If the seed-mash is fermented by the associated organisms, first lactic and then butyric acid bacteria increase considerably: the fungi normally present as spores and which, in consequence, germinate rather slowly, multiply rapidly at this stage and soon suppress all the other forms of life. The above are distinctly cases of natural selection. The bacteria mentioned above produce acids and gases which discourage most other forms of life. The fungi thrive best in slightly acid media and are favoured by the presence of organic acids, which they readily oxidise to carbon dioxide: so they multiply rapidly after the first few days and in doing so, suppress all the other micro-organisms. In a like manner, treatment with antiseptics to isolate the specific associates of seeds also results only in the selection of the more resistant organisms. Non-sporers are readily killed while those which occur as spores are only partially destroyed by the antiseptic. Spores of fungi and certain soil bacteria persist even after prolonged treatment with the commoner antiseptics and are thus the only organisms to be isolated after the treatment. The bacteria and the fungi thus found in association with the seed cannot be described as being in symbiotic relation with the latter. They are only artificial selections under the conditions of the experiments and are no more symbiotic with the seeds than those organisms which were killed by the antiseptics. Nor can such organisms be described as being specific because the flora isolated after treatment with antiseptics were not consistent and included a large number of bacteria and fungi.

No evidence was obtained to indicate the presence of specific micro-organisms inside any germinating seeds. Such bacteria and fungi as were isolated from seeds of which the outer coats had been previously sterilised by heat and antiseptics were (*a*) generally some of the commoner soil forms and (*b*) not consistent in composition. This observation in conjunction with the previous one to the effect that the seed-coat is generally only partially sterilised as the result of the treatments mentioned above leads to the inference that the

organisms isolated by such methods were essentially those which were left on the seed-coats and which had originally been derived from the soil.

A similar argument could be applied to show that the thermo-resistant, Fitz-type organisms isolated from seed-mashes are (*a*) only artificial selections and (*b*) not specific to any of the seeds as they are actually derived from the soil. It is no doubt possible that there are several varieties of such organisms occurring under identical conditions and that some are physiologically more active than others: it is also possible that the biochemical activity of such organisms and their efficiency in converting starchy materials into acetone, butyl alcohol and other allied products are largely determined by the chemical composition of the seed or the tuber with which they are associated and on which they are raised. But the fact remains that they are normal inhabitants of the soil and bear no specific relationship to any of the seeds or tubers from which they are isolated.

A certain amount of evidence in favour of symbiosis between seeds and associated bacteria has been adduced by some of the earlier workers from their studies on the germination of seeds of which the outer coats were sterilised by heat or antiseptics. Such investigations did not however include tests to determine whether (*a*) the sterilisation of the seed-coat was normally complete, and (*b*) the treatments adopted for sterilisation did not have any direct effect on germination. The present studies have shown that none of the commoner methods of sterilisation leads to complete elimination of all the associated micro-organisms. The germinating seed is left with a few resistant forms which in the absence of the other micro-flora necessary to maintain the biological equilibrium may adversely affect the seedling. Moreover, the seed-coat always retains traces of the antiseptic used for the sterilisation: such chemicals being highly toxic to the seedlings, the growth of the latter is rapidly affected. Thus traces of bleaching powder, copper sulphate, mercuric chloride, formaldehyde, or toluene though without much effect on the associated micro-organisms, are either deadly to the embryo or highly poisonous to the seedlings. The effects persist after adding suspensions of the mixed soil-flora, or sufficient soil extract to restore the original conditions. Thus it would be seen that the effects attributed to the removal of associated bacteria were really due to the toxic action of antiseptics used for sterilising the seed-coats. Further proof of this fact was obtained from the observation that the treated seeds from which the last traces of the antiseptic were carefully removed, germinated just as satisfactorily as those from which the associated micro-organisms were not removed.

Addition of specific organisms or products of microbial activity did not help either germination or the development of the seedling.

In fact, some of them proved to be inimical to the growth of the seedling. The presence of minute quantities of lactic acid in all germinating seeds, even after sterilisation of the seed-coat shows that the acid is a normal product of seed-metabolism and is unconnected with the presence of micro-organisms.

Treatment with bacterial or plant nutrients, or chemical poisons do not generally evoke similar response from the seeds and the associated micro-organisms; conditions favouring one form of life may be distinctly inimical to the other. The same series of experiments also showed that even when the response was found to be similar, the seeds and the associated bacteria behaved in the same way as they would have done if they had occurred independently of each other.

It may perhaps be argued that the nitrogen-fixers associated with the root nodules of *Leguminoseæ* are specific symbionts of those seeds. Recent work has however shown that such is not the case. The nodule organisms are no doubt present in the soil in which the legume has been established for some years, or into which the organisms have been introduced by inoculation. The bacteria may even occur in large numbers on the seed-coats : but they do not bring about germination or help in the early nourishment of the seedling. They move towards the roots and form the nodules only after the seedlings have established themselves. It therefore follows that the nodule bacteria are in active symbiotic association with the growing plants and not with the seeds.

The results of the present investigation show conclusively that under normal conditions, the seeds and the associated micro-organisms are physiologically unrelated to each other. The occurrence of the latter on seed-coats is merely due to the inevitable accident of finely divided soil being scattered by wind as dust. The prominence of a single organism or group of organisms under certain artificial conditions would be merely cases of selection and thus bear no relation to the natural association of such forms with the seed.

SUMMARY.

1. A study of the nature and the distribution of micro-organisms associated with a large number and variety of seeds showed that the former are all derived from the soil and that none of them is specific to any of the seeds.

2. Treatment with heat or antiseptics does not sterilise the seed-coat completely, but leads to the selection of one or more resistant forms, which are not generally specific to the seeds from which they are isolated.

3. There is no direct evidence to show that living organisms are present inside healthy seeds.

4. Sterilisation of the seed-coat does not in any way affect either germination or development of the seedling, provided the last traces of the antiseptic used for the purpose are removed.

5. Addition of active cultures of specific organisms does not help germination, but may prove inimical to the growth of the seedling under certain adverse conditions.

6. The presence of micro-organisms, within or without the seed, is not necessary for the production of lactic acid which is formed in minute quantities inside all germinating seeds.

7. Treatment with nutrients, stimulants or toxics does not generally evoke a similar response from seeds and associated bacteria.

8. Under natural conditions, seeds and soil micro-organisms are inevitable associates which are physiologically unrelated and live independently of each other.

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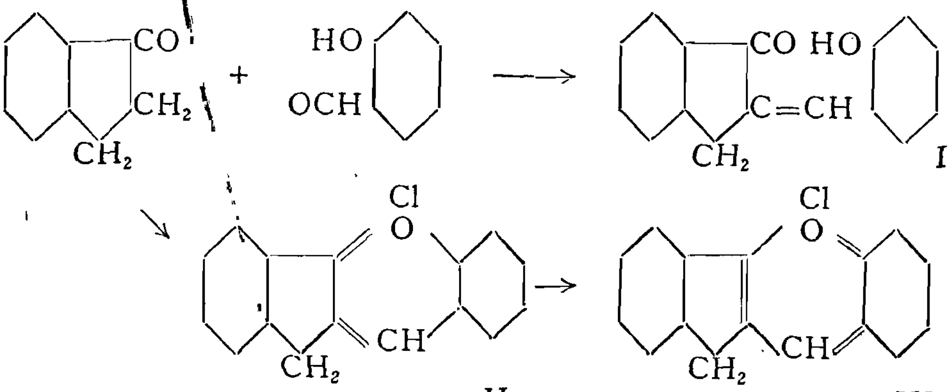
THE REACTIVITY OF DIMETHYLDIHYDRORESORCIN. Part I. CONDENSATION WITH AROMATIC ALDEHYDES.

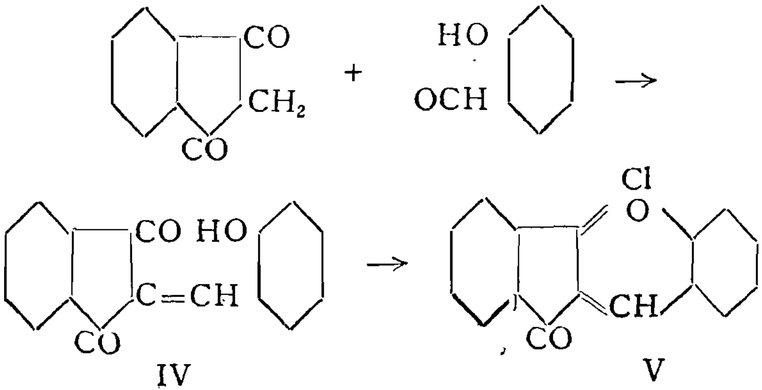
By Gopâl Chandra Chakravarti, Haripada Chattopadhyaya and Prafulla Chandra Ghosh.

Monoketohydrindene reacts with salicylaldehyde in presence of hydrogen chloride yielding indenobenzopyranol anhydrochloride (Perkin, Robinson and Turner, *J.C.S.*, 1908, **93**, 1085) :—



I II III

The strong colour of the compound, and various other considerations led the authors to assume that its constitution is best represented by the orthoquinonoid formula (III), involving a rearrangement of the double bonds in formula (II). Sastry and Ghosh later (*J.C.S.*, 1915, **107**, 1442) obtained keto-indenobenzopyranol anhydrochloride (V) from diketohydrindene and salicylaldehyde,

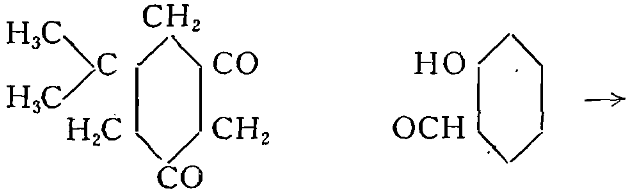


IV V

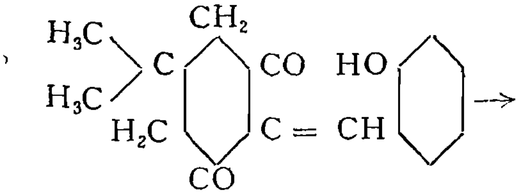
They think that the assumed shifting of double bonds to the benzene nucleus is unnecessary in view of the fact that compound IV and its acetyl derivative are coloured. The present work was undertaken with the object of arriving at a decision between the two views.

Many compounds containing the CO-CH_2-group have in recent years been shown to yield pyranol derivatives (*J.C.S.*, 1918, **113**, 444; *ibid.*, 1919, **115**, 817; 812 *et seq.*): but as none of these contains a hydroaromatic ring the orthoquinonoid arrangement is possible on both sides. If a hydroaromatic compound containing the CO-CH_2-group be condensed with *o*-hydroxyaldehydes, the resulting pyranol derivatives can have orthoquinonoid arrangement on one side only, namely, that containing the benzene nucleus.

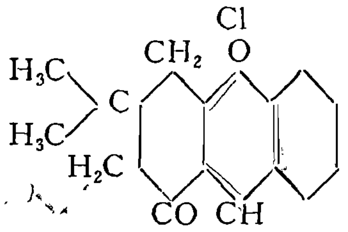
Dimethyldihydroresorcin is a well-crystallised, reactive cyclic compound containing two CO-CH_2-groups; it was therefore expected to yield pyranol derivatives by condensation with *o*-hydroxyaldehydes.



VI

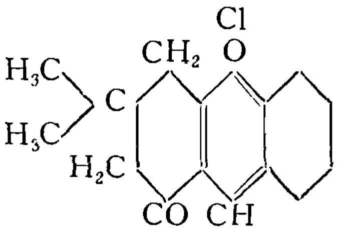


VII



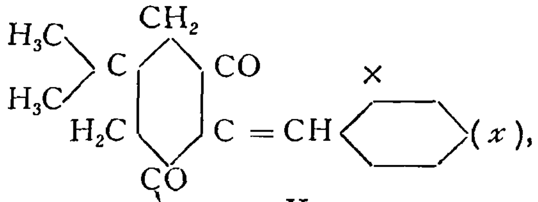
VIII

In the above formula double bonds are attached to the carbon atoms of a hexahydrobenzene nucleus and therefore do not form what is called orthoquinonoid arrangement. Now if the pyranol derivatives so produced be strongly coloured the double bonds must have been shifted to the benzene nucleus to produce the orthoquinonoid arrangement. The pyranol derivative obtained from salicylaldehyde is scarlet, so the double bonds must have been shifted during the course of the reaction and the compound has the formula :—



IX

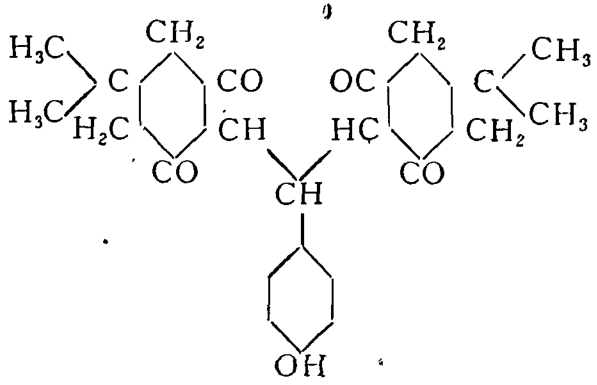
If this is true, compounds of the type,



X

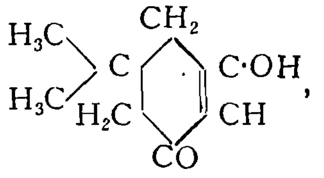
should either be colourless or be very lightly coloured. Attempts were therefore made to synthesise compounds of the type (X) by condensing dimethyldihydroresorcin with benzaldehyde and substituted benzaldehydes in presence of alkali, but in none of these cases was a benzylidene derivative obtained. On the other hand, mono- and diketo-hydrindenes and substances of allied nature are known to yield benzylidene derivatives (cf. Kipping, *J.C.S.*, 1894, **65**, 498; Perkin and Robinson, *ibid.*, 1907, **91**, 1073 *et seq.*) of structures similar to (I) and (IV) almost exclusively by the same treatment. The experiments were conducted under various conditions, (1) by boiling the aldehyde and the resorcin dissolved in absolute alcohol, (2) by adding a few drops of alkali to the alcoholic solution, (3) by employing aqueous alcoholic potash and also (4) in presence of aqueous caustic soda and sodium carbonate solutions; but in every case two molecules of dimethyldihydroresorcin were found to condense with one molecule of

aldehyde producing compounds of the benzamarone type. With *p*-hydroxybenzaldehyde the triphenylmethane derivative (XI), is



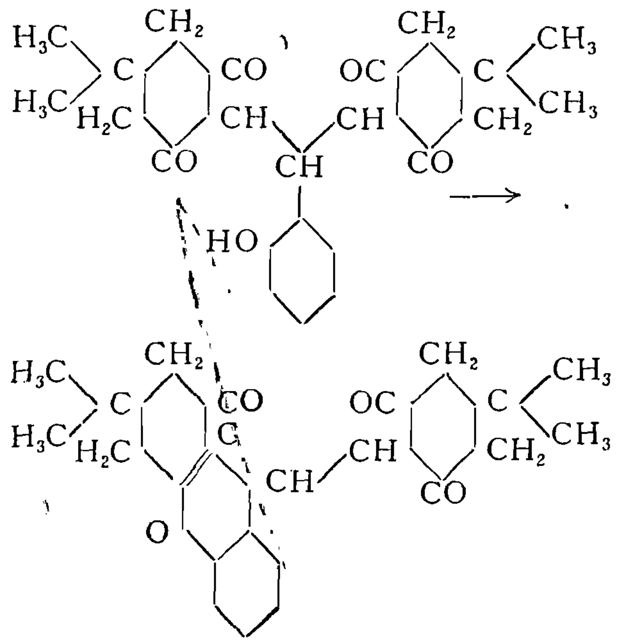
XI

formed; similar compounds have been obtained by the action of benzaldehyde, anisaldehyde, vanillin, piperonal, para- and meta-nitrobenzaldehydes, 3 : 4-dihydroxybenzaldehyde and cinnamaldehyde, on the ketone. The recent investigation of compounds (aldimethones) of dimethyldihydroresorcin with aldehydes occurring in plant products by Vorländer (*Z. anal. Chem.*, 1929, **77**, 321–327), Zeffmann and Pines (*Bull. Wagner Free Inst.*, 1929, **4**, 15–16) and Klein and Zinser (*Mikrochem.*, 1929, Pregl Fest, 204–234) also lends support to our observation that aromatic aldehydes react with dimethyldihydroresorcin in the manner suggested. The foregoing facts lead us to the conclusion that the reactions of dimethyldihydroresorcin itself are best explained if we assume that the latter has the constitution,



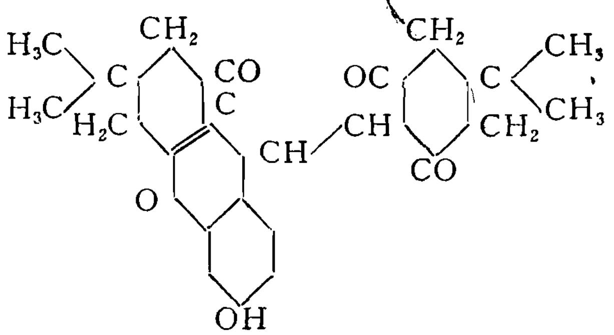
and not the customary formula (VI) by which it is usually represented; but we shall discuss this point in connection with further experiments which are about to be completed. The behaviour of salicylaldehyde is of special interest; as in the above cases, two molecules of the ketone condense with one molecule of the aldehyde, but the salical derivative

which is undoubtedly first formed loses a further molecule of water yielding the pyran derivative (XII) :—



XII

In the same way 2 : 4-dihydroxybenzaldehyde reacts with dimethyldihydroresorcin forming the compound (XIII) :—



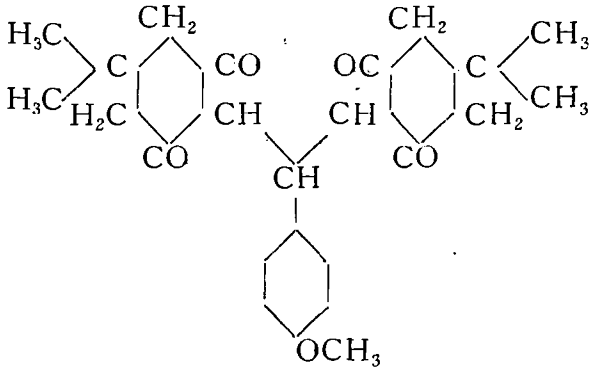
XIII

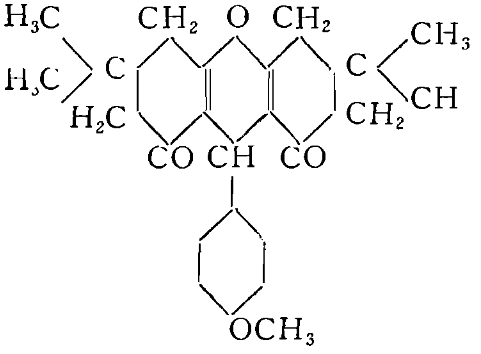
Evidence for such a supposition is (1) the results of analysis, which do not agree with any other possible formula, and (2) although the substance is colourless in the solid state and also in chloroform or alcohol solution, it is orange-yellow in the molten state and in glacial acetic acid solution, owing probably to the formation of a weak oxonium

salt. The formation of 3 : 3′-diphenylacetylenedibenzo*spiro*pyran and of diphenylacetylenedi-β-naphtha*spiro*pyran from diphenyl*cyclo*pentanone and salicylaldehyde and 2-naphthol-1-aldehyde (Ruhemann and Levy, *J.C.S.*, 1913, **103**, 553–54), although not exactly analogous, may be cited as examples of the ready anhydride formation in which the hydroxyl group of salicylaldehyde and of naphtholaldehyde takes part.

Having failed to obtain the benzylidene derivatives by direct synthesis, the hydrolysis of the anhydrochloride (VIII) was attempted. It was expected that ortho-hydroxybenzylidenedimethyldihydroresorcin (VII) would be formed by such a treatment. The anhydrochloride was found to be remarkably stable towards alkali however; and no appreciable hydrolysis took place even by boiling it with concentrated alcoholic potash for six hours.

The benzamarone type of compounds mentioned above, on boiling with acetic anhydride or on heating their solution in strong sulphuric acid to about 100° undergo a xanthene-ring condensation. Anisaldehyde compound gives 2 : 7-tetramethyl-4 : 5-diketo-*p*-methoxyphenyloctahydroxanthene (XIV) :—

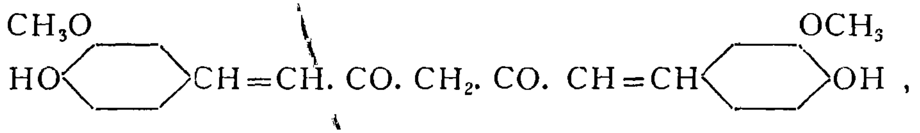




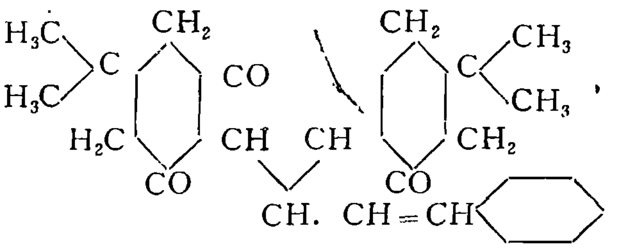
XIV

Compounds obtained from *p*-hydroxybenzaldehyde, vanillin and para- and meta-nitrobenzaldehyde with dimethyldihydroresorcin have also yielded similar hydrogenised phenylxanthene derivatives. The latter compounds are also formed along with the benzamarone derivatives by boiling the alcoholic solutions of the aldehyde and resorcin, the quantities of the products varying according to the duration of heating; and they are formed exclusively if such solutions in absolute alcohol are saturated with hydrogen chloride gas and allowed to stand. Hydrogenised phenylxanthene rings of this type are unknown; the nomenclature to be adopted for the compounds will be the same as for ordinary phenylxanthene derivatives.

It has been found by Ghosh (*J.C.S.*, 1919, **115**, 292) that curcumin, a diolefinic-β-diketone having the formula,



on bromination gives first a monobromo-derivative. He thinks that bromination attacks the labile hydrogen atom in preference to the double bonds. Cinnamaldimethyldihydroresorcin contains double bonds and labile hydrogen atoms. Bromination of this compound was therefore regarded as interesting; the cinnamal derivative (XV),

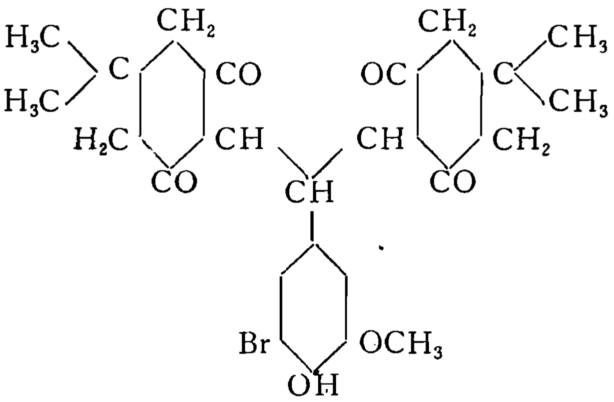


XV

does in fact give first a monobromo-derivative.

Crossley (*J.C.S.*, 1914, **105**, 165) has found that bromodimethyldihydroresorcins undergo a remarkable transformation to bromoxylenols. Some of the benzamarone type of compounds have been brominated to see whether these compounds undergo the same sort of transformation. The vanillin compound on bromination with 1½

molecules and 3 molecules of bromine gives only a monobromo-derivative whereas anisaldehyde compound with 3 molecules of bromine gives a mixture of tri- and tetrabromo-derivatives; with 1½ molecules of bromine also a mixture of two compounds both containing bromine is obtained, one of which has a very low halogen content (5·87 per cent.). Nothing can be said as to the nature of that compound. In these compounds the bromine atom can enter the benzene nucleus as well as the hydroaromatic. The monobromovanillin compound being the simplest for investigation has been studied in detail. If the bromine atom entered the benzene nucleus the monobromo-derivative must have the following constitution :—

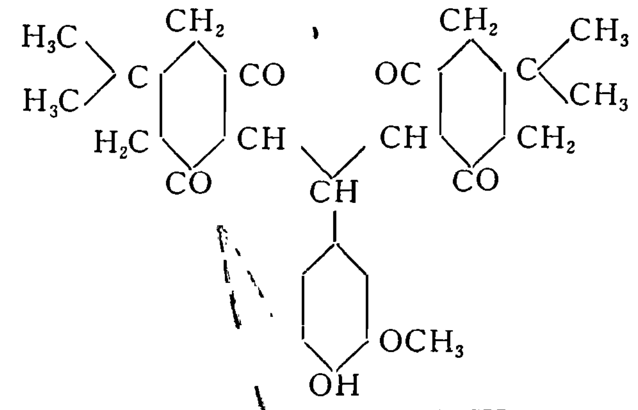


XVI

The phenolic hydroxyl group directs the bromine atom into the ortho-position because the para-position is blocked, and this compound should be identical with the compound prepared from 5-bromovanillin and dimethyldihydroresorcin, which is the case. However it is difficult to understand why bromination with 3 molecules of bromine also produces the monobromo-derivative, the hydroaromatic ring being altogether unattacked. Tri- and tetrabromo-derivatives obtained from the anisaldehyde compound may improbably have all the bromine atoms in the benzene nucleus, or some of the bromine atoms might have attacked the hydroaromatic ring. In any case there is a progressive bromination which seems to militate against our experience with the vanillin compound. We are making experiments to elucidate this remarkable behaviour of very closely related compounds.

Further interest attached to the preparation of the above compounds is their expected physiological activity. The alkaline condensation product with vanillin possesses hydroaromatic rings

containing the $CO\text{-}CH_2$-group as well as the hydroxy- and methoxy-groups in the ortho-position to one another in a benzene nucleus :—



XVII

It was therefore thought probable that it would be a good internal antiseptic, and in fact it inhibits the growth of the typhoid bacillus ; but its therapeutic value must be ascertained by clinical experiments.

EXPERIMENTAL.

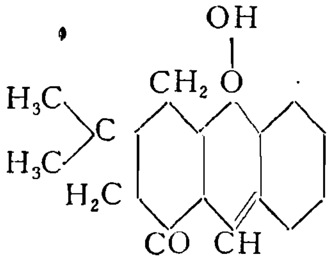
Dimethyldihydroresorcin was prepared according to Komppa's method (*Ber.*, 1899, **32**, 1421) ; the 20 hours' heating recommended by him need not be continuous, but after pouring the condensation product of malonic ester and mesityl oxide into baryta solution, 7–8 hours' continuous heating is necessary to secure the yield obtained by Komppa.

Salicylaldehyde and Dimethyldihydroresorcin.

2-Dimethyl-4-ketotetrahydrobenzopyranolanhydrochloride (VIII).— Dimethyldihydroresorcin (1 g.) and salicylaldehyde (0·8 g.) were dissolved in the minimum quantity of alcohol and saturated with a rapid current of dry hydrogen chloride during ice-cooling. The solution became dark red and overnight deposited scarlet needles ; these were filtered, washed with absolute alcohol and remained scarlet after drying in a vacuum desiccator. The substance does not melt below 290°, but becomes pale yellow ; it is sparingly soluble in almost all solvents and so cannot be recrystallised. In contact with air-moisture it becomes brown owing to conversion into the free base, and this rapid decomposition impairs the combustion results. The compound is stable in presence of hydrogen chloride, which is removed from it

with water on heating (Found: C, 68·5; H, 6·2; Cl, 6·0-10·5. $C_{15}H_{15}O_2Cl$ requires C, 68·9; H, 5·7; Cl, 13·5 per cent.).

Free Base,



The anhydrochloride was warmed with a large excess of pyridine and after cooling was treated with dilute sodium acetate solution, filtered, washed with water and dried. The pale brown substance is transparent under the microscope, but has no definite geometrical form; being sparingly soluble in all the common solvents it cannot be recrystallised. It does not melt below 290°, and even when heated on the platinum foil it burns without fusion (Found: C, 72·8; H, 6·7. $C_{15}H_{16}O_3$ requires C, 73·7; H, 6·5 per cent.). This compound is also obtained by boiling the anhydrochloride for six hours with alcoholic potash; the ring is not broken.

o-*Hydroxybenzaldimethyldihydroresorcinanhydride* (XII).—Dimethyldihydroresorcin (7 g.) and salicylaldehyde (5·6 g.) were mixed with 30 c.c. of water, and heated on the water-bath for two hours with caustic potash (5·6 g.) dissolved in 11 c.c. of water. The orange-red solution was then cooled, poured into water, filtered and acidified with dilute hydrochloric acid. The product was oily at first, but crystallised from alcohol in milk white, rhombic prisms and melts at 206° after shrinking at 200°: the yield after one crystallisation was 6·2 grams (Found: C, 75·5; H, 6·5. $C_{23}H_{26}O_4$ requires C, 75·4; H, 7·1 per cent.). The compound dissolves in dilute caustic alkalis; the solution in chloroform and alcohol is colourless, and in glacial acetic acid orange-yellow.

Acetyl derivative.—One gram of the above compound, 8 c.c. of acetic anhydride and 3 drops of pyridine were heated on the sandbath for about an hour, the clear solution depositing colourless cubes on cooling; recrystallisation from alcohol gave colourless cubes melting at 188°, the yield being 0·7 gram (Found: C, 73·2; H, 6·8. $C_{25}H_{28}O_5$ requires C, 73·5; H, 6·8 per cent.).

Benzoyl derivative.—One gram of the substance was dissolved in pyridine with a few c.c. of 10 per cent. caustic soda; benzoyl chloride was then added producing an oil which solidified completely after a day. Crystallisation from alcohol gave colourless leaves melting at

152–53°, the yield being 0·7 gram (Found: C, 76·95; H, 7·0. $C_{30}H_{30}O_5$ requires C, 76·6; H, 6·4 per cent.).

Bromo-derivative.—The substance (1·2 g.) was almost completely dissolved in warm chloroform (50 c.c.), when 6 c.c. of a chloroform solution of bromine (0·5 c.c. bromine in 12 c.c. of chloroform) was added. On the first addition of 2 c.c. the whole of the substance went into solution, and fifteen minutes after all had been added the chloroform was evaporated by blowing air. The emulsion was dissolved in rectified spirit and poured into water, when the oil soon became semi-solid. Crystallisation from absolute alcohol gave colourless, clear-cut needles, melting and decomposing at 194° (Found: Br, 15·83. $C_{23}H_{25}O_4Br, C_2H_6O$ requires Br, 16·35 per cent.).

Nitroso-derivative.—The substance (0·4 g.) dissolved in boiling glacial acetic acid was gradually treated with potassium nitrite (0·4 g.); when all had dissolved the solution was cooled and on dilution with water yellow leafy crystals separated melting at 100–3°. It is soluble in acetic acid, alcohol, benzene and acetone, and dissolves in dilute caustic alkali with yellow colour, alcoholic ferric chloride producing an orange-red coloration (Found: N, 4·8. $C_{23}H_{24}O_6N_2, 6H_2O$ requires N, 5·26 per cent.).

Phenylhydrazone.—The substance (1·2 g.) was dissolved in hot acetic acid and treated with phenylhydrazine (1·2 g.) dissolved in acetic acid. After warming for a few minutes water was added, the solid crystallising from absolute alcohol in colourless needles melting at 237–41°.

Resorcylaldehyde and Dimethyldihydroresorcin.

2:4-*Dihydroxybenzaldimethyldihydroresorcinanhydride* (XIII).—Resorcylaldehyde (1 g.) and dimethyldihydroresorcin (1 g.) were dissolved in alcohol and treated with potash (1·5 g. dissolved in 3 c.c. of water), when the colour became deep yellow, and after boiling under reflux for about 45 minutes, brownish-red. On dilution with water the filtrate was very slightly acidified with hydrochloric acid, avoiding excess, and the solid crystallised from dilute alcohol; on recrystallisation it melted at 225–6° with gas evolution, the yield being 0·3 gram of colourless prismatic needles. It dissolves in alkali with slight yellow green fluorescence (Found: C, 72·3; H, 6·4. $C_{23}H_{26}O_5$ requires C, 72·2; H, 6·8 per cent.).

p-*Hydroxybenzaldehyde and Dimethyldihydroresorcin.*

4-*Hydroxybenzaldimethyldihydroresorcin* (XI).—*p*-Hydroxybenzaldehyde (1 g.) and dimethyldihydroresorcin (1 g.) were dissolved in alcohol and heated on the water bath under reflux for half-an-hour

with potash (0·8 g.) dissolved in water (1·5 c.c.). After pouring into water and filtration, the filtrate was acidified with dilute hydrochloric acid, the emulsion so produced being again dissolved in alkali and precipitated with dilute hydrochloric acid. Crystallisation from dilute alcohol gave colourless octagons melting at 184° to a yellow liquid (yield, 1·2 g.). It dissolves readily in caustic alkalis with yellow-red fluorescence (Found: C, 71·7; H, 6·6. $C_{23}H_{28}O_5$ requires C, 71·8; H, 7·2 per cent.).

2 : 7-*Tetramethyl*-4 : 5-*diketo*-p-*acetoxyphenyloctahydroxanthene*.—The above compound (0·5 g.) with acetic anhydride (4 c.c.) and 3 drops of pyridine was gently boiled on the sand-bath for 45 minutes. The crystals formed on cooling separated from spirit in long colourless silky needles melting at 208–9°; the yield was 0·25 g. (Found: C, 73·4; H, 6·3. $C_{25}H_{28}O_5$ requires C, 73·5; H, 6·8 per cent.).

Protocatechuic aldehyde and Dimethyldihydroresorcin.

3 : 4-*Dihydroxybenzaldimethyldihydroresorcin*.—Equimolecular quantities of protocatechuic aldehyde and dimethyldihydroresorcin were dissolved in alcohol, poured into excess of concentrated aqueous potash, and gently boiled on the water-bath under reflux for an hour. The red colour darkened, and the solution was then diluted with water and filtered. Alternate acidification, dissolution in alkali and reprecipitation with dilute hydrochloric acid gave a black tarry mass, from which spirit extracted colourless prisms melting at 145° with decomposition. It dissolves in dilute caustic soda solution with orange-pink fluorescence (Found: C, 66·1; H, 7·2. $C_{23}H_{28}O_6,H_2O$ requires C, 66·02; H, 7·17 per cent.).

Piperonal and Dimethyldihydroresorcin.

3 : 4-*Methylenedioxybenzaldimethyldihydroresorcin*.—Dimethyldihydroresorcin (1·4 g.) and piperonal (1·5 g.) were dissolved in the minimum of alcohol, and with potash (1 g.) in water (2 c.c.) boiled on the water-bath under reflux for half-an-hour; poured into water and filtered, the filtrate was acidified with dilute hydrochloric acid, the resulting oil becoming partly solid after several days. The white solid on crystallisation from alcohol melted at 131–34°. The semi-solid portion was repeatedly dissolved in glacial acetic acid and precipitated with water, but even then did not solidify completely. It was left for several days, rubbed with water in a mortar, and the collected solid dried and freed from gummy matter by alcohol. Crystallisation from rectified spirit gave large, colourless needles melting at 136–37° (Found: C, 69·06; H, 7·0. $C_{24}H_{28}O_6$ requires C, 69·9; H, 6·7 per cent.).

Benzaldehyde and *Dimethyldihydroresorcin*.—Benzaldehyde (3 g.) and dimethyldihydroresorcin (4 g.) were heated with absolute alcohol on the water-bath for 3 hours when most of the alcohol was distilled and the residue diluted with water, the precipitated solid being fractionally crystallised from alcohol. The first fraction on recrystallisation from the same medium melted at 193° and was found to be 2 : 7-*tetramethyl*-4 : 5-*diketophenyloctahydroxanthene* (Found: C, 78·6; H, 7·19. $C_{23}H_{26}O_3$ requires C, 78·8; H, 7·4 per cent.). The last fraction on crystallisation from alcohol melted at 175–76° and proved to be *benzaldimethyldihydroresorcin* (Found: C, 75·13; H, 7·8. $C_{23}H_{28}O_4$ requires C, 75·0; H, 7·6 per cent.).

Anisaldehyde and Dimethyldihydroresorcin.

4-*Methoxybenzaldimethyldihydroresorcin*.–Dimethyldihydroresorcin (5 g.) and anisaldehyde (5 g.) in alcohol were heated under reflux on the water-bath for half-an-hour with potash (2 g.) in water (4 c.c.). The orange solution was diluted with water, filtered and acidified with dilute hydrochloric acid; the oil crystallised overnight and was recrystallised from spirit, forming colourless, lustrous cubes melting at 139–40°, the yield being 5·6 grams (Found: C, 71·75; H, 7·3; OCH_3, 7·17. $C_{24}H_{30}O_5$, requires C, 72·3; H, 7·5; OCH_3, 7·7 per cent.).

2 : 7-*Tetramethyl*-4 : 5-*diketo*-p-*methoxyphenyloctahydroxanthene* (XIV).—The above compound (0·5 g.) with acetic anhydride (4 c.c.) and 3 drops of pyridine was heated on the sand-bath for 45 minutes, and the colourless crystals obtained on cooling recrystallised from a large volume of alcohol giving lustrous crystals melting at 243–46°, the yield being 0·3 gram (Found: C, 74·9; H, 7·2; OCH_3, 7·2. $C_{24}H_{28}O_4$ requires C, 75·7; H, 7·3; OCH_3, 8·1 per cent.).

Vanillin and Dimethyldihydroresorcin.

4-*Hydroxy*-3-*methoxybenzaldimethyldihydroresorcin* (XVII).—Dimethyldihydroresorcin (3 g.) and vanillin (3·3 g.) dissolved in the minimum of alcohol, with potash (3 g.) in water (6 c.c.) were heated on the water-bath under reflux during half-an-hour. A solid appeared and redissolved, the solution being orange. Poured into water and filtered, the filtrate after acidification with dilute hydrochloric acid deposited colourless prisms which when recrystallised from dilute alcohol melted at 195° after softening slightly at 189°. It dissolves in alkali with deep orange-red fluorescence, and the yield was 3·2 grams (Found: C, 69·3; H, 6·7; OCH_3, 7·2. $C_{24}H_{30}O_6$ requires C, 69·5; H, 7·2; OCH_3, 7·4 per cent.).

2 : 3-*Tetramethyl*-4 : 5-*diketo*-p-*acetoxy*-m-*methoxyphenyloctahydroxanthene*.—The above compound (0·5 g.) with acetic anhydride (5 c.c.)

and 3 drops of pyridine was heated on the sand-bath during 45 minutes and poured into water, the oil soon solidifying. It crystallised from dilute alcohol in long, colourless needles melting at 148–49°, the yield being 0·4 gram (Found: C, 67·4; H, 6·2; OCH_3, 6·68. $C_{26}H_{30}O_6$, H_2O requires C, 67·5; H, 5·87; OCH_3, 6·7 per cent.).

Bromination of p-*methoxybenzal* and p-*hydroxy*-m-*methoxybenzal-dimethyldihydroresorcin.*—Bromine (1½ mol.) was dissolved in chloroform and gradually added to the chloroform solution of the *p*-methoxy-compound. There was copious evolution of hydrogen bromide, and when the bromine colour had almost disappeared the chloroform was vaporised. The residue dissolved in boiling alcohol deposited colourless prismatic needles melting at 193–198° (Found: Br, 6·8. The monobromo-derivative requires Br, 16·7 per cent.).

The product was therefore fractionally crystallised from spirit several times yielding two compounds melting at 200–3° and 232°, the latter being the less soluble. The former contains 5·5 per cent. of bromine and the compound melting at 232° also shows a low bromine content.

Using the double quantity of bromine and proceeding as before, a microcrystalline powder was obtained. It is soluble in alcohol, benzene, acetone and acetic acid, but as it is not crystallisable from any of these solvents, it was fractionally precipitated from alcoholic solution with water. The first fraction crystallised from spirit in prismatic needles melting at 207° (0·2 g.) and the second fraction, also from spirit, in needles which melted at 173°. This is the *tribromo-derivative* being more soluble in alcohol than the tetrabromo-compound (Found: Br, 34·95. $C_{24}H_{27}O_5Br_3$, $3H_2O$ requires Br, 34·83 per cent.); the compound melting at 207° being the *tetrabromo-derivative* (Found: Br, 44·94. $C_{24}H_{26}O_5Br_4$ requires Br, 44·81 per cent.).

From the *p*-hydroxy-*m*-methoxy-compound and bromine (1½ mol.) under similar conditions the solid product crystallised from dilute alcohol in colourless cubes melting at 211–13° (Found: C, 57·6; H, 5·5; Br, 15·94. $C_{24}H_{29}O_6Br$ requires C, 58·4; H, 5·8; Br, 16·22 per cent.). Using the double quantity of bromine (3 mols.) the same compound melting at 211–13° was obtained, and is identical with 5-bromo-4-hydroxy-3-methoxybenzaldimethyldihydroresorcin.

5-*Bromo*-4-*hydroxy*-3-*methoxybenzaldimethyldihydroresorcin* (XVI). 5-Bromovanillin (2 g.) and dimethyldihydroresorcin (1·5 g.) dissolved in alcohol with potash (1·5 g.) in water (3 c.c.) were heated on the water-bath under reflux during half-an-hour, poured into water and filtered. The rose-pink filtrate was acidified with dilute hydrochloric

acid and the turbid liquid was decanted from a crystalline substance which on recrystallisation from alcohol was found to be 5-bromovanillin. The turbid liquid deposited a semi-solid mass after two days, and thrice crystallised from dilute alcohol melted at 210–13°. It is found by the mixed melting point to be identical with the foregoing compound from *p*-hydroxy-*m*-methoxybenzaldimethyldihydroresorcin.

p-*Nitrobenzaldehyde and Dimethyldihydroresorcin.*

4-*Nitrobenzaldimethyldihydroresorcin.*—*p*-Nitrobenzaldehyde (1 g.) and dimethyldihydroresorcin (1 g.) in alcohol with potash (0·4 g.) in water (1 c.c.) were heated under reflux during half-an-hour on the water-bath. The preliminary orange-red colour changed to deep-red on heating. The solution was poured into water and filtered, acidified with dilute hydrochloric acid and the crystalline precipitate recrystallised from spirit, forming beautiful, colourless needles melting at 188–90°, the yield being 1·4 grams (Found: N, 3·31. $C_{23}H_{27}O_6N$ requires N, 3·36 per cent.).

2 : 7-*Tetramethyl*-4 : 5-*diketo*-p-*nitrophenyloctahydroxanthene.*—The above compound (2 g.) in concentrated sulphuric acid was heated on the water-bath during 45 minutes, then poured into a large volume of water and the precipitated solid crystallised from alcohol; recrystallisation gave needles melting at 222°, the yield being 1·2 grams (Found: N, 3·67. $C_{23}H_{25}O_5N$ requires N, 3·5 per cent.).

m-*Nitrobenzaldehyde and Dimethyldihydroresorcin.*

3-*Nitrobenzaldimethyldihydroresorcin.*—*m*-Nitrobenzaldehyde (1 g.) and dimethyldihydroresorcin (1 g.) treated as above deposited a solid which was recrystallised from spirit in prismatic needles melting at 186–88°, the yield being 1·2 grams (Found: N, 3·8. $C_{23}H_{27}O_6N$ requires N, 3·36 per cent.).

Cinnamic aldehyde and Dimethyldihydroresorcin.

Cinnamaldimethyldihydroresorcin (XV).—Cinnamic aldehyde (2 g.) and dimethyldihydroresorcin (2 g.) in alcohol with potash (0·8 g.) in water (1·6 c.c.) were heated on the water-bath during half-an-hour. After dilution with water the filtrate was acidified with dilute hydrochloric acid, and the viscous product, after solidification, was crystallised from dilute alcohol. It formed lustrous needles melting at 202°, unchanged on recrystallisation, the yield being 1·3 grams (Found: C, 76·4; H, 7·07. $C_{25}H_{30}O_4$ requires C, 76·1; H, 7·61 per cent.). The *bromo-derivative* was recrystallised from alcohol, forming colourless needles melting at 180–81° with decomposition (Found: Br, 19·1. $C_{25}H_{29}O_3Br$ requires Br, 17·89 per cent.).

Action of acetic anhydride.—The substance with acetic anhydride and a few drops of pyridine was heated on the sand-bath during 45 minutes, poured into water, and the solidified oil crystallised from dilute alcohol until the melting point reached 147–48°; the yield was very poor.

Condensation with piperonal using hydrogen chloride.—Dimethyldihydroresorcin (1·4 g.) and piperonal (1·5 g.) were dissolved in the minimum of alcohol, and submitted to a current of dry hydrogen chloride under ice-cooling to saturation. After two days the violet solution was poured into water when an oil separated which solidified after three days; powdered in a mortar under water, the dried substance was partially dissolved in boiling alcohol and filtered. Crystals remaining on the filter paper recrystallised from alcohol in prismatic needles, and finally melted at 214°, being 2 : 7-*tetramethyl*-4 : 5-*diketo*-3′; 4′-*methylenedioxyphenyloctahydroxanthene* (Found: C, 72·83; H, 6·9. $C_{24}H_{26}O_5$ requires C, 73·1; H, 6·6 per cent.).

Condensation with m-*nitrobenzaldehyde.*—*m*-Nitrobenzaldehyde and dimethyldihydroresorcin treated in the same way gave 2 : 7-*tetramethyl*-4 : 5-*diketo*-m-*nitrophenyloctahydroxanthene*, which crystallised from spirit in colourless cubes melting at 169°, the yield being 40 per cent. (Found: N, 3·7. $C_{23}H_{25}O_5N$ requires N, 3·5 per cent.).[1]

[1] A preliminary report of this paper appeared in the Indian Science Congress Abstracts, 1929, p. 22.

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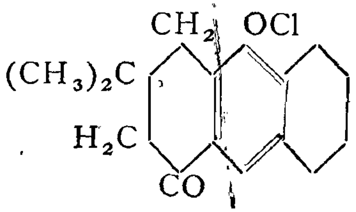
THE REACTIVITY OF DIMETHYLDIHYDRORESORCIN. PART II. BEHAVIOUR TOWARDS *ORTHO*-NITRO- AND *ORTHO*-AMINOBENZALDEHYDES.

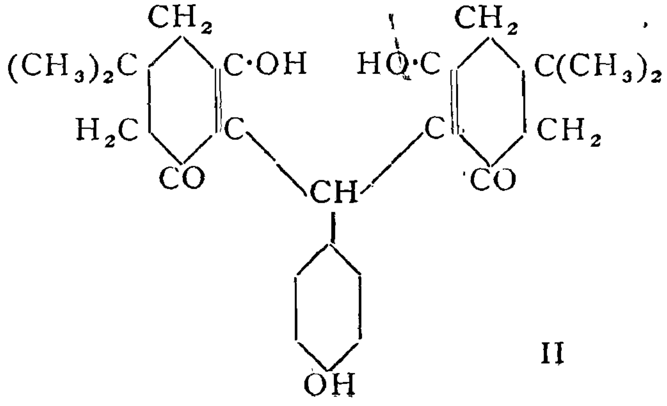
By B. H. Iyer and G. C. Chakravarti.

Chakravarti, Chattopadhyaya and Ghosh (Indian Science Congress Abstracts, 1929, 22), in condensing dimethyldihydroresorcin with aromatic aldehydes, have shown the various forms of ring closure taking place; the reactive methylene group between the two keto-groups gives dimethyldihydroresorcin great synthetic importance. This substance on condensation with *o*-hydroxybenzaldehyde in presence of dry hydrogen chloride gave 2-dimethyl-4-keto-tetrahydrobenzopyranol anhydrochloride (I),



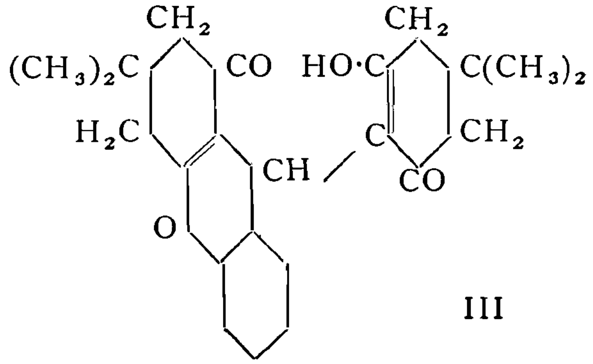
I

but condensation with salicylaldehyde in alcoholic potash gave compounds of the benzamarone type. Here two molecules of dimethyldihydroresorcin combined with one molecule of the aldehyde, whereas with hydrogen chloride only molecular proportions reacted. With *p*-hydroxybenzaldehyde, 4-hydroxybenzaldimethyldihydroresorcin (II) was obtained,



II

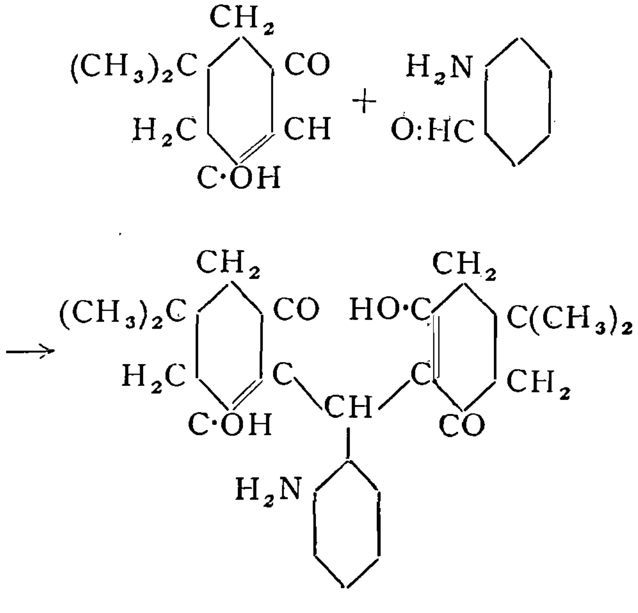
elimination of only one molecule of water taking place; but when the HO-group is in the *ortho*-position as in salicylaldehyde, two molecules of water are eliminated giving *o*-hydroxybenzaldimethyldihydroresorcin anhydride (III).

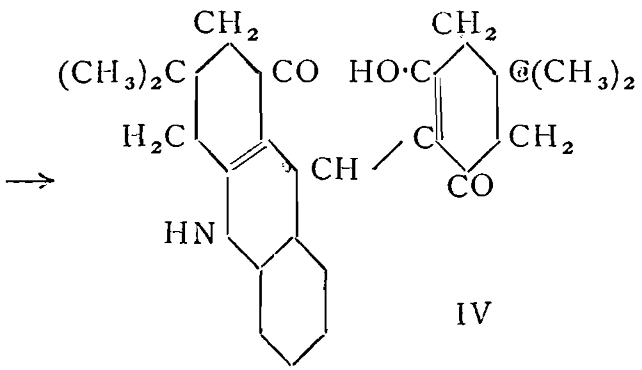


III

Owing to this interesting behaviour of the *o*-hydroxybenzaldehyde, it was thought that *o*-aminobenzaldehyde might give a dihydropyridine derivative of the type (IV).

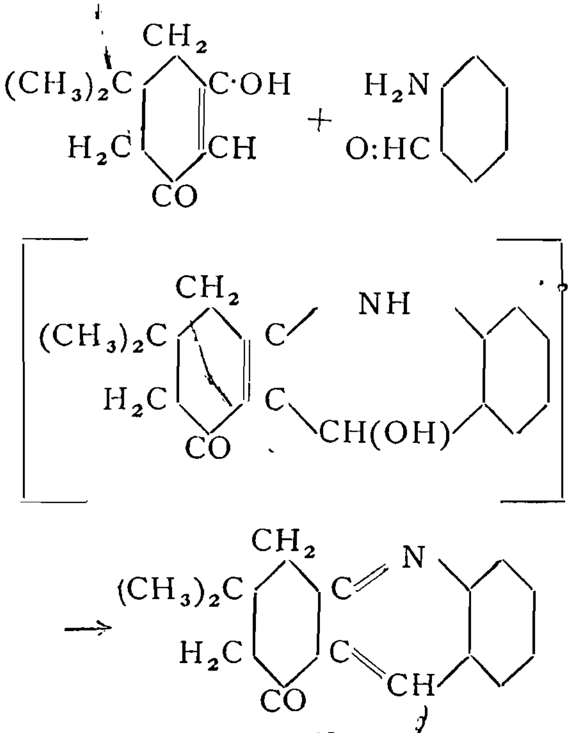
This product may be of great importance since it is likely to possess alkaloidal properties, having a reduced pyridine ring fused with hydroaromatic rings. The reaction was expected to take the following course:





IV

From the outset it was observed that *o*-aminobenzaldehyde and its derivatives behaved towards dimethyldihydroresorcin quite differently from the other aldehydes, only molecular proportions reacting. When dimethyldihydroresorcin was condensed with *o*-aminobenzaldehyde in alcoholic potash, only molecular proportions were involved and 1-keto-3-dimethyltetrahydroacridine (V) was formed by elimination of two molecules of water. The course of the reaction can be explained as follows :

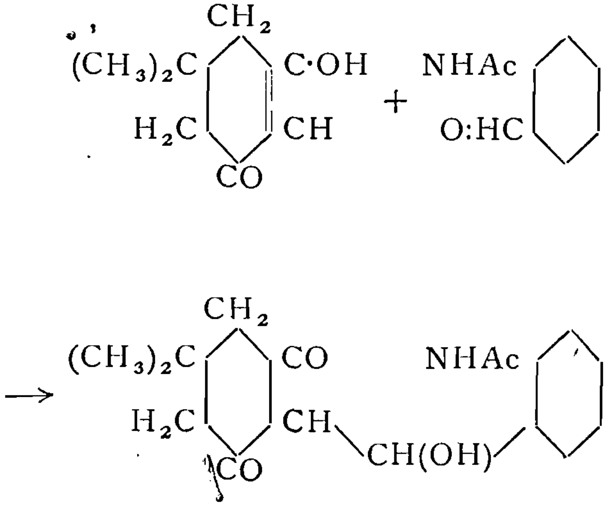


V

Elimination of water from the intermediate benzhydrol (not isolated) between the CH (OH) and NH-groups prevents a second molecule of dimethyldihydroresorcin from reacting.

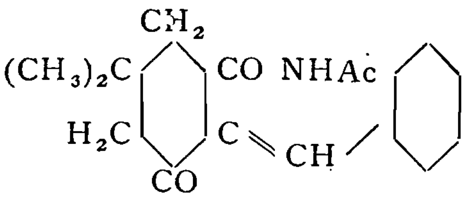
The above constitution for the product (V), namely, 1-keto-3-dimethyltetrahydroacridine, follows from, (1) Its tertiary basic properties indicated by non-formation of a nitroso- and acetyl derivative; (2) Negative colour test for the hydroxyl group with ferric chloride; (3) Formation of a methiodide, chloroplatinate and picrate. Moreover, when oxidised with concentrated nitric acid, it yields acridinic acid (2 : 3-quinolinedicarboxylic acid). With phenylhydrazine the product (V) yields the phenylhydrazones of dimethyldihydroresorcin and *o*-aminobenzaldehyde. Such behaviour has been observed in similar compounds by Sastry and Ghosh (*J.C.S.*, 1915, **107**, 1444) and in the case of oximes by Perkin and Robinson (*J.C.S.*, 1907, **91**, 1088)

To ascertain whether the NH_2-group was responsible for the non-formation of a pyridine derivative, it was protected by acetylation, but when dimethyldihydroresorcin was condensed with *o*-acetaminobenzaldehyde in alcoholic potash, the same acridine derivative was formed, owing to hydrolysis of the acetyl group. In absolute alcohol also only molecular proportions of the reactants combined; benzamarone condensation did not take place probably owing to steric hindrance by the acetamino-group in the ortho-position. The product was 2 : 6-diketo-4-dimethyl-2′-acetaminohexahydrobenzhydrol (VI), giving the acridine derivative (V) on hydrolysis with alcoholic potash.



VI

When the benzhydrol compound was heated at 105–110° for 15 to 20 hours, two molecules of water were eliminated with formation of 2-acetaminobenzylidenedimethyldihydroresorcin (VII).

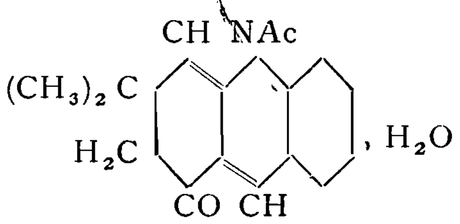


VII

The benzylidene derivative is colourless thus confirming the conclusion of Chakravarti, Chattopadhyaya and Ghosh (*J. Ind. Inst. Sci.*, 1931, **14A**, 141) that compounds of this structure should be colourless or very lightly coloured, although they were not synthesised. The *ortho*-quinonoid formula (I) for 2-dimethyl-4-ketotetrahydrobenzopyranol anhydrochloride was then suggested on account of the remarkable chromophoric character of the substance. This view necessitates that products similar to (VII) having a pair of double bonds in the *ortho*-position of a reduced benzene ring should be colourless. The observation therefore is strikingly in agreement with the theoretical forecast.

The next procedure was to condense the two substances in presence of a dehydrating agent and with a large excess of dimethyldihydroresorcin. Using fused zinc chloride, a product was obtained melting at 203–204° and having the composition $C_{17}H_{19}O_3N$. Even with six molecular proportions of dimethyldihydroresorcin, both dry (i.e. by fusion) and in absolute alcohol, combination of more than the molecular proportion never took place, definitely showing the impossibility of linking two molecules with one of the *o*-aminobenzaldehyde or its acetyl derivative, directly.

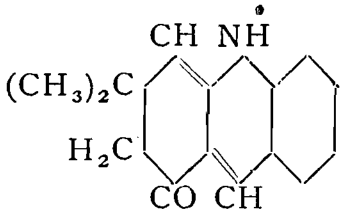
The compound $C_{17}H_{19}O_3N$ might be represented by either of the formulæ (VII) and (VIII),



VIII

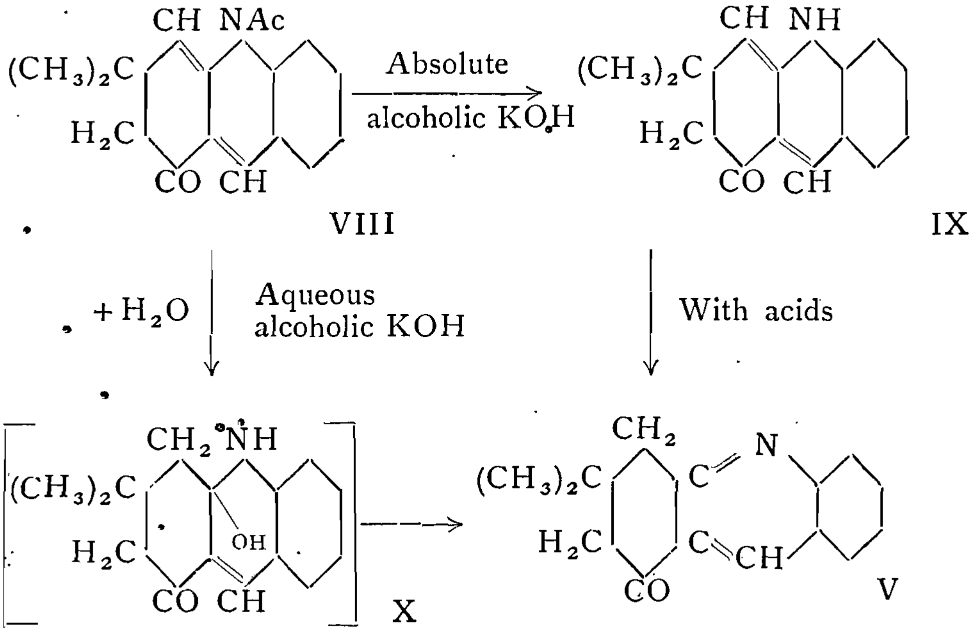
Whereas analytical results point to (VII), the authors prefer the formula (VIII) from a study of the chemical properties. When hydrolysed, both compounds are expected to give the acridine derivative

(V), and this is produced by aqueous alcoholic potash; but with potash and absolute alcohol, an isomeride melting at 245–250° (with decomposition) was formed. This is 2-dimethyl-4-ketotetrahydrobenzoquinoline (IX),



IX

changing into the acridine compound spontaneously during many days, more readily in contact with water and instantaneously on dissolution in acids and precipitation by alkali. The mechanism of the hydrolysis can best be explained as following from 2-dimethyl-4-keto-tetrahydro-*N*-acetylbenzoquinoline (VIII),

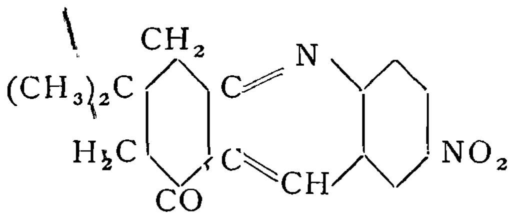


With aqueous alcoholic potash, one molecule of water is added to form the intermediate compound (X), not isolated; elimination of water would then give the acridine derivative (V).

The high melting hydrolysis product (IX) when treated with hydrochloric acid and sodium nitrite also gives the acridine compound, because the acid effects transformation before a nitroso-derivative can be formed.

The benzhydrol and the benzylidene compounds (VI and VII) when dissolved in concentrated sulphuric acid, warmed for half-an-hour and precipitated by water gave the benzoquinoline derivative (VIII). Thus the benzhydrol when heated loses the water of crystallisation and then another molecule of water to form the benzylidene compound, which when treated with sulphuric acid yields the benzoquinoline derivative by loss of another molecule of water; on the other hand, if the benzhydrol is treated with sulphuric acid, the benzoquinoline complex is directly formed without the intermediate benzylidene compound.

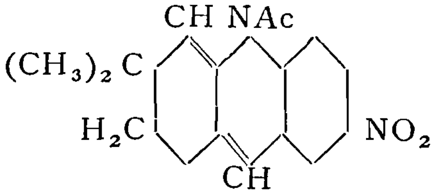
Similar condensations were conducted with 5-nitro-*o*-acetaminobenzaldehyde; when this was condensed with dimethyldihydroresorcin in alcoholic potash, 1-keto-3-dimethyl-7-nitrotetrahydroacridine (XI), m.p. 82–83° was obtained:



XI

In absolute alcohol no union took place, the materials being recovered almost quantitatively.

When 5-nitro-*o*-acetaminobenzaldehyde and dimethyldihydroresorcin were condensed in presence of fused zinc chloride without a solvent 2-dimethyl-4-keto-7-nitro-*N*-acetyltetrahydrobenzoquinoline (XII) was produced,

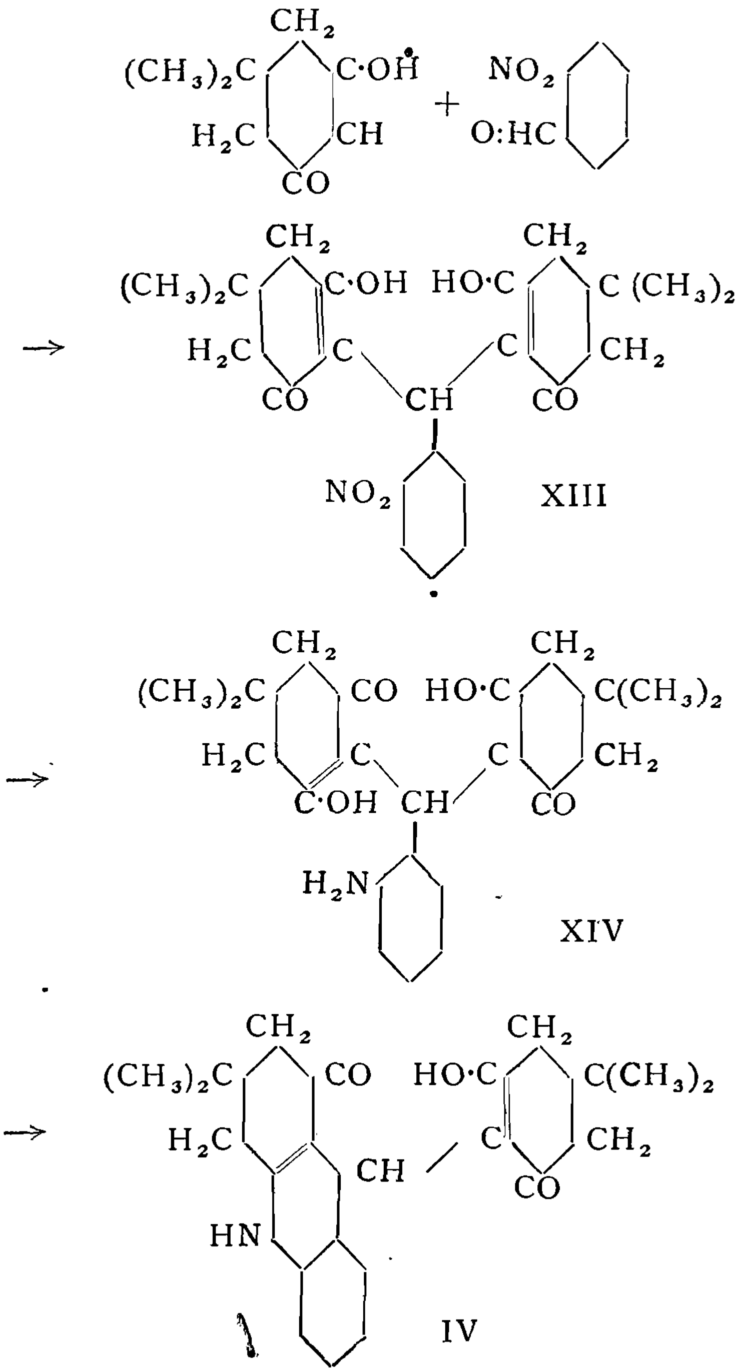


XII

and on hydrolysis with aqueous alcoholic potash readily gave the nitroacridine compound (XI).

When it was found that no benzanthrone condensation could be brought about directly with *o*-aminobenzaldehyde or its acetyl

derivative, dimethyldihydroresorcin was condensed with *o*-nitrobenzaldehyde with the intention of proceeding as follows :

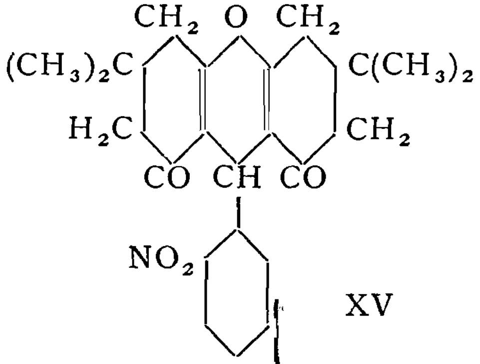


XIII

XIV

IV

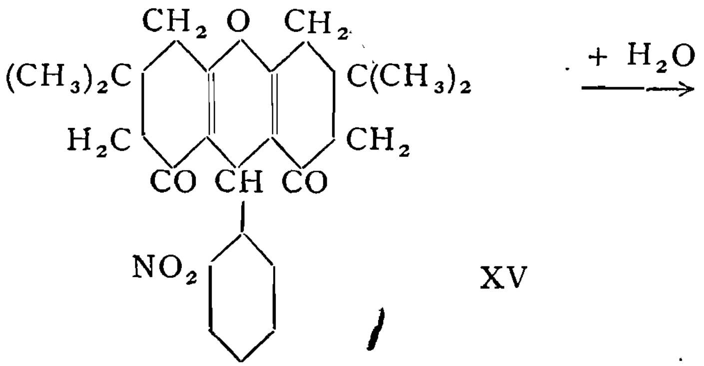
The 2-nitro-benzaldimethyldihydroresorcin (XIII) melts at 195° and gives a cherry-red coloration with ferric chloride, showing the presence of hydroxyl groups. When the condensation was conducted in absolute alcohol for a longer period, a mixture of compound (XIII) with 2 : 7-tetramethyl-4 : 5-diketo-*o*-nitrophenyloctahydroxanthene (XV), m.p. 253–254°, was obtained:



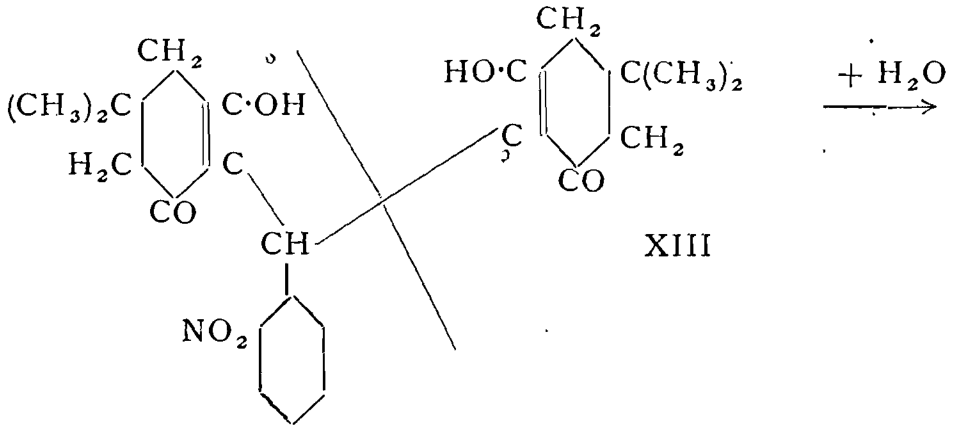
XV

This compound does not give a coloration with ferric chloride, and is separated by fractional crystallisation from alcohol, in which the benzal compound (XIII) is more soluble. When this is treated with concentrated sulphuric acid, it is converted quantitatively into the xanthene, which being more stable than the benzal compound was employed in the reduction experiments.

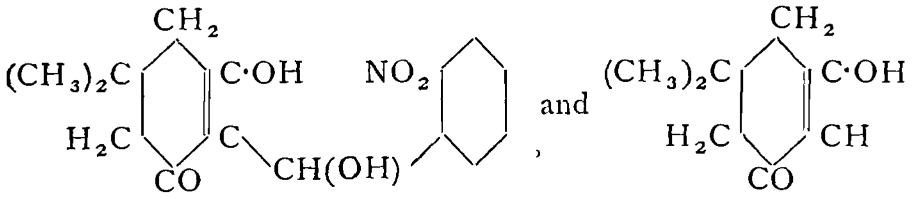
Reduction of (XV) was conducted with zinc dust in acetic acid containing a small quantity of hydrochloric acid. The product was a mixture of three or four compounds, the one melting at 246–249° being phototropic. The acridine derivative arises from hydrolysis of the molecule by hydrochloric acid, because in experiments without this acid the substance remained unchanged. First the xanthene derivative (XV) is converted into the benzal derivative (XIII) which is disrupted, forming the nitrobenzhydrol compound (XVI), which after reduction is transformed into the acridine compound:



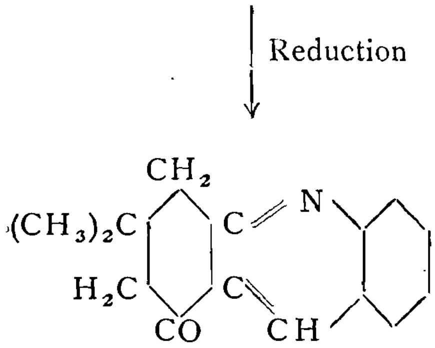
XV



XIII



XVI



V

It is suspected that the product melting at 246–249° obtained by the reduction of the xanthene derivative (XV) is the one sought, and its investigation will be undertaken when a sufficient quantity has been prepared.

EXPERIMENTAL.

Preparation of *o*-aminobenzaldehyde by the method of Friedländer (*Ber.*, 1882, **15**, 2572) presented several difficulties. *o*-Nitrobenzaldehyde was first reduced by tin and glacial acetic acid to anthranil which was converted into the anthranil-mercuric chloride compound and reduced to aminobenzaldehyde by ferrous sulphate and ammonia. The process was tedious owing to the large quantity of steam distillate collected for extraction after saturation with common salt in both the stages of reduction, and the difficulty was augmented by the unstable nature of anthranil and *o*-aminobenzaldehyde. The former is an oil which changes into a solid melting above 200°, and *o*-aminobenzaldehyde, which ordinarily melts at 39°, simply on keeping changes into a compound melting above 250°. Both polymerides have been described in the literature.

Direct reduction of *o*-nitrobenzaldehyde by means of ferrous sulphate and ammonia was then tried according to Bamberger and Demuth (*Ber.*, 1901, **34**, 1330) who stated that 8 g. of *o*-nitrobenzaldehyde required only 55 c. c. of concentrated ammonia, whereas Bamberger (*Ber.*, 1927, **60**, 319) in a later communication found that 85 c.c. were needed. The present authors also required 80 to 85 c. c. to make the escaping vapours alkaline, which is the test for completion of the reduction. Until 55 c. c. of ammonia are added, the black precipitate of ferrous hydroxide changed after some time into yellow ferric hydroxide, but the escaping vapours were not alkaline; further addition of ammonia was controlled by the test of the escaping vapours and not by change in colour of the precipitate. Regulation of the quantity of ammonia is essential to the success of this preparation.

o-Acetaminobenzaldehyde was obtained according to Friedländer and Ghoring (*Ber.*, 1884, **17**, 456–461), while 5-nitro-*o*-acetaminobenzaldehyde was prepared by the method of Cohn and Springer (*Monatsh.*, 1903, **24**, 87–100).

Dimethyldihydroresorcin and o-*Aminobenzaldehyde.*

1-*Keto*-3-*dimethyltetrahydroacridine* (V).—*o*-Aminobenzaldehyde (3·7 g.) and dimethyldihydroresorcin (4 g.) were dissolved in the minimum quantity of absolute alcohol, and heated under reflux over a water-bath for 2 to 3 hours with caustic potash (2 g.) in water (4·4 c.c.). The mixture was then poured on ice, stirred, acidified with dilute hydrochloric acid, filtered and the filtrate kept overnight. Concentrated caustic soda produced a colourless crystalline precipitate, which was filtered, freed from dimethyldihydroresorcin by hot water and crystallised from alcohol and water, in faint, yellowish-green plates, m.p. 117° (Yield 3·4 g.). It crystallised from petrol ether (b.p. 40–60°) in needles (Found :

C, 79·78; H, 7·07; N, 6·48. $C_{15}H_{15}ON$ requires C, 80·00; H, 6·6; and N, 6·20 per cent.). The same compound was obtained by the alkaline hydrolysis of 2:6-diketo-4-dimethyl-2′-acetaminohexahydrobenzhydrol and 2-acetaminobenzylidenedimethyldihydroresorcin.

The picrate after crystallisation from alcohol melted at 197–198°, sintering at 184° (Found: N, 12·35; $C_{15}H_{15}ON$, $C_6H_2(NO_2)_3OH$ requires N, 12·36 per cent.).

The chloroplatinate was recrystallised from alcohol (Found: Pt, 22·40; Cl, 24·48. $(C_{15}H_{15}ON)_2PtCl_6$ requires Pt, 22·67; Cl, 24·77 per cent.).

The methiodide was best prepared by heating the substance sealed with large excess of methyl iodide in a boiling water-bath during over four hours. When cold, the large needles of methiodide were recrystallised from alcohol, m.p. 224–225°. The compound on standing decomposed slowly, hence an accurate estimation of the iodine content could not be made (Found: I, 35·72. $C_{15}H_{15}ON$,MeI requires I, 34·7 per cent.)

Oxidation did not take place with potassium permanganate in neutral and alkaline solutions, either cold or hot; the acridine compound was therefore dissolved in concentrated nitric acid (8·4 parts, sp. gr. 1·42) and heated under reflux over a water-bath until evolution of nitrous fumes ceased, when the liquid was heated by an open flame until colourless. Water was added and the precipitate formed after some delay was filtered, washed thoroughly and recrystallised from hot water. The melting point rose to 272° indicating the formation of the aminocarboxylic acid produced on heating acridinic acid (Graebe and Caro, *Ber.*, 1880, **13**, 100). Analysis also proved that it was acridinic acid, but the yield was very poor (Found: N, 6·06. $C_{11}H_7O_4N$ $2H_2O$ requires N, 6·45 per cent.).

Dimethyldihydroresorcin and o-*Acetaminobenzaldehyde.*

2 : 6-*Diketo*-4-*dimethyl*-2′-*acetaminohexahydrobenzhydrol* (VI).—*o*-Acetaminobenzaldehyde (0·4 g.) and dimethyldihydroresorcin (0·35 g.) in the minimum quantity of absolute alcohol were heated under reflux on a water-bath for 45 minutes and poured into ice-water, the white precipitate weighing 0·6 g. The substance was freed from dimethyldihydroresorcin by boiling water and the crystals from alcohol and water contained one molecule of water of crystallisation. It is insoluble in ether and melts at 153–154° (Found: C, 63·5; H, 7·62; N, 4·35. $C_{17}H_{21}O_4N$, H_2O requires C, 63·55; H, 7·17; N, 4·36 per cent.).

2-*Acetaminobenzylidenedimethyldihydroresorcin.*—The colourless benzhydrol (0·2637 g.) was heated at 105–110° for over 15 hours, becoming brownish and losing 10·5 per cent. The calculated percentage of water of crystallisation for the formula $C_{17}H_{21}O_4N,H_2O$, is 5·6 per cent., and for loss of two molecules of water, 11·2. The 2-acetaminobenzylidenedimethyldihydroresorcin (VII) thus formed melts at 197–199° (Found: C, 72·56; H, 7·81. $C_{17}H_{19}O_3N$ requires C, 71·6; H, 6·7 per cent.).

2-*Dimethyl-4-keto-N-acetyl-tetrahydrobenzoquinoline* (VIII).—*o*-Acetaminobenzaldehyde (2·3 g.) and dimethyldihydroresorcin (2 g.) dissolved in the minimum quantity of absolute alcohol with freshly fused zinc chloride (5 g.) were heated under reflux for about 3 hours, poured on ice, and the white precipitate crystallised from dilute acetic acid, forming long needles, m.p. 203–204°. It is very soluble in alcohol and is insoluble in ether, petrol ether, water and chloroform; slightly soluble in benzene, toluene and xylene; soluble in acetone, amyl alcohol and methyl alcohol. The same compound is obtained from the benzhydrol and the benzylidene compounds described above (Found: C, 71·42; 71·47; H, 7·26; 7·38; N, 5·16. $C_{17}H_{17}O_2N$, H_2O requires C, 71·58; H, 6·67; N, 4·92 per cent.). It retains the molecule of water of crystallisation even when heated at a high temperature for a very long period. Hydrolysis with aqueous alcoholic potash gives 1-keto-3-dimethyltetrahydroacridine, the colour changing through yellow and dull red to deep pink.

2-*Dimethyl-4-ketotetrahydrobenzoquinoline* (IX) was obtained by heating 2-dimethyl-4-keto-*N*-acetyltetrahydrobenzoquinoline dissolved in absolute alcohol with solid caustic potash under reflux for about three hours and immediately pouring on ice; the brown precipitate was crystallised from absolute alcohol, m.p. 245–250° with decomposition (Found: C, 79·82; H, 7·04. $C_{15}H_{15}ON$ requires C, 80·00; H, 6·6 per cent.).

Dimethyldihydroresorcin and 5-Nitro-o-Acetaminobenzaldehyde.

1-*Keto-3-dimethyl-7-nitrotetrahydroacridine* (XI).—5-Nitroacetaminobenzaldehyde (1 g.) and dimethyldihydroresorcin (0·8 g.) were dissolved in the minimum of absolute alcohol and heated under reflux for 2–3 hours with caustic potash (0·4 g.) in water (1 c. c.). When cooled, poured into ice-water, acidified with dilute hydrochloric acid and filtered, a small quantity of dimethyldihydroresorcin was recovered. The filtrate was then treated with concentrated alkali, giving a yellow precipitate (0·5 g.) which was filtered, washed and crystallised from alcohol and water, m.p. 82–83° (Found: N, 10·81. $C_{15}H_{14}O_3N$ requires N, 10·33 per cent.).

2-Dimethyl-4-keto-7-nitro-N-acetyltetrahydrobenzoquinoline (XII)—5-Nitro-2-acetaminobenzaldehyde (1 g.) and dimethyldihydroresorcin (0·8 g.) in the minimum of absolute alcohol with fused and powdered zinc chloride (3 g.) were heated under reflux on a water-bath for 2–3 hours, cooled and poured into ice-water; the light yellow precipitate (0·5 g.) was filtered, washed several times with boiling water and crystallised from alcohol, m.p. 171° (Found: N, 9·21. $C_{17}H_{16}O_4N_2$ requires N 8·97 per cent.).

Dimethyldihydroresorcin and o-Nitrobenzaldehyde.

Nitrobenzaldimethyldihydroresorcin (XIII) from dimethyldihydroresorcin (1 g.) and *o*-nitrobenzaldehyde (1 g.) in the minimum of absolute alcohol with caustic potash (0·4 g.) in water (1 c.c.), refluxed for 3–4 hours, was crystallised from alcohol and melted at 195°. It is insoluble in ether, petrol ether and benzene but very soluble in chloroform, acetone and methyl alcohol (Found: C, 66·73; H, 6·60; N, 3·89. $C_{27}H_{27}O_6N$ requires C, 66·81; H, 5·58; N, 3·4 per cent.). A small quantity of a high melting (253–255°) substance was also obtained.

2:7-Tetramethyl-4:5-diketo-o-nitrophenyloctahydroxanthene (XV).—Dimethyldihydroresorcin (5 g.) and *o*-nitrobenzaldehyde (4 g.) in absolute alcohol were heated under reflux for 4 to 5 hours when most of the alcohol was distilled; the diluted residue gave a precipitate (6 g.) which was washed with water and ether to remove unchanged materials. It melted at 190–195° and contained principally the benzal compound; so was dissolved in concentrated sulphuric acid, warmed for an hour and then poured into much water, when a quantitative yield of the xanthene derivative was obtained. This was crystallised from alcohol, m.p. 253–254° (Found: C, 69·64, H, 6·78; N, 4·23. $C_{23}H_{25}O_5N$, requires C, 69·86; H, 6·33; N, 3·54 per cent.).

Reduction of the xanthene derivative (XV).—The xanthene derivative (6 g.) was well mixed with zinc dust (4 g.) in a mortar, transferred to a conical flask and adding 90 c.c. of glacial acetic acid, this was boiled under reflux for half-an-hour; 100 c.c. of concentrated hydrochloric acid was added, and the heating continued for another 15 minutes, when the mixture was filtered hot and diluted with much water. Ammonia was added until the precipitated zinc hydroxide dissolved, and a slimy yellow precipitate formed. Extraction with ether and removal of the solvent left about 3 g. of yellow resinous substance which on retreatment with a small quantity of ether gave an ether-insoluble precipitate. Crystallisation from alcohol and water gave a light yellow substance, m.p. 246–249°, changing to orange-red on exposure to sunlight; the original colour was restored in darkness. As only a very small amount of this substance was obtained, it could

not be analysed. The ether-soluble portion after driving off ether is repeatedly extracted with petrol ether and the product crystallised from alcohol and water, m.p. 116–117°. It was found to be identical with the acridine derivative. There was, in addition, a third product which was originally insoluble in ether.

The foregoing method of reduction was found to be superior to (1) alcoholic ammonium sulphide at 100° in a sealed tube: (2) iron and hydrochloric acid: (3) tin and acetic acid: and (4) tin and hydrochloric acid.

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